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Molecular Pathology of Viral Respiratory Diseases

Geoffrey A. Land

Introduction

Virology has long been the gold standard by which advances in molecular biology and methodology have been measured.1 As new molecular tools have been developed, new viruses or variants of older, established taxons have been described. Recent advances in genetic sequencing and amplification technologies were pivotal in detecting and describing the two newest agents with a tropism for the respiratory system, severe acute respiratory syndrome (SARS) and avian influenza virus,2–4 both of which have the potential to be pandemic agents with a high mortality and morbidity rate. The rapid development of specific molecular tests led to effective public health measures to be put in place to successfully quarantine these agents thus far. The identification of these two new agents underscores the fact that the major cause of nonbacterial epidemics in history has been viruses with a predilection for the respiratory system. The classic example is the global “Spanish flu” pandemic of 1918, attributed to causing the deaths of 20–40 million people within 1 year, a mortality rate greater than that recorded for World War I and the 4 worst years of the Black Plague (AD 1347–1351) combined.4–7

The recognition of viruses as entities and as potential agents of infection has occurred only within a little more than the past 115 years.8,9 The Russian bacteriologist Dimitrii Ivanovsky presented evidence of small infective agents that could pass through unglazed porcelain filters that retained bacteria. Martinus Beijerinck, in 1898, hypothesized that these “filterable agents” could cause tobacco mosaic disease in a nondiseased plant. Similar filterable agents were described in 1901 by Walter Reed and James Carroll as the probable etiology of yellow fever, and soon thereafter there were descriptions of similar agents that caused malignancies in birds or that could eradicate bacteria.8 The actual isolation and propagation of human viruses was a difficult process and languished behind the advances made in animal, plant, and bacterial viruses.9 The major stumbling block in the development of animal virology was finding a satisfactory milieu or substrate for culturing human viruses. Initial success at growing viruses used animals or eggs, making human virology beyond the scope of everything but research laboratories. However, the development in the 1950s of eukaryotic cell culturing techniques and cell lines that sustained viral growth and propagation enabled the rapid development of diagnostic virology.8

The first human respiratory virus, influenza virus, was described in 1933 by Wilson Smith, Christopher Andrews, and Patrick Laidlaw.4 Interest in human influenza research had become stimulated over the devastation left by the Spanish flu pandemic, but it was not until a suitable host was found that the virus could be studied in depth.8 Studies to date have determined that this virus can undergo degrees of spontaneous mutation of some of the major proteins on its outer surface, the hemagglutinin (H) and neuraminidase (N) proteins, at a fairly regular rate.10 Minor changes to these proteins (antigenic drift) lead to localized epidemics, whereby the antibodies stimulated in response to the previous strain are not as protective as they should be. There is, however, enough immunologic memory in the population that the virus cannot find a large group of unexposed individuals and cause a pandemic (worldwide epidemic). More profound changes in the composition of the H and N molecules can also occur (antigenic shift) in which the global immunologic memory of these antigens has been lost or never stimulated and a pandemic ensues.11,12 The reason for the current concern over SARS and avian flu is based not only on the rate of change of the H and N molecules over the last 100 years in the case of influenza viruses11 but also on the similarity both of these virus genomes have to their respective animal and avian counterparts1–3,11,13 as well as recent evidence pointing to the human influenza flu virus family evolving from a crossover that occurred during the domestication of animals approximately 8,000 years ago.14
The intimate relationship of viral structure and extent and severity of influenza virus infections in humans has served as a benchmark for describing the relationship between the various macromolecules produced by other viruses and the course and pathology of their infections. \(^{4,7,10,15-17}\) This chapter focuses on recent discoveries in the macromolecular structures of respiratory viruses and their contribution in establishing infection and concomitant pathology. This includes sections on viral structure, genomics, replication, the molecular events leading to respiratory tropism and pathology, and a brief description of the viruses involved.

**General Principles**

**Structure and Invasion**

Viruses are nonliving macromolecular complexes made up of proteins and either DNA or RNA that, upon gaining access to the host cell’s energy and reproductive system, effectively redirect host cell metabolism and synthetic capabilities in order to replicate and transmit progeny virus (release). \(^{9,18}\) Their general structure consists of an outer protein shell (capsid) composed of subunits, which are single folded polypeptides that link together as the basic structural unit (protomer). \(^{8}\) These basic structural units may consist of one specific peptide type or multiple types, each with various functions relative to attaching and gaining entry into the cell as they condense to form the outer shell or, in the case of some viruses, that have condensed around a protein–genomic complex called the nucleocapsid. \(^{8,9}\) In the case of enveloped viruses, these protomers may also become surface structures (capsomers, peplomers) such as spikes, projections, or knobs that give a virus its characteristic shape and appearance in electron micrographs. Finally, the tertiary folding of single polypeptides and their quaternary interaction and folding as they condense around the genome (packaging) impart a characteristic symmetry to the resulting macromolecular complex. \(^{8}\) Viral symmetry is defined as either icosahedral or helical, and its geometry is directed by the steric interaction of folded structural units. In addition to their combined structure protecting the genome, the outer proteins function in binding the virus to specific receptors on the cell, \(^{20,21}\) helping to prepare the virus for entry into the host cells by having protease and/or nuclease activities, interaction with the host cell membranes (budding) to develop the outer envelope, \(^{8,22}\) or, in some cases, eventually inducing fusion with host cell membranes. \(^{23-25}\)

Viruses enter host cells by (1) uptake through attachment to specific receptors or ligands (attachment) on the surface membrane of the target cells (receptor-mediated endocytosis) \(^{8,23,26-28}\) or (2) by being absorbed into the cell by the cellular vesicular/endosome system (pinocytosis, fluid phase endocytosis, absorption). \(^{20,29-31}\) Receptor-mediated or ligand entry is facilitated by the attachment of a specific epitope on the protein outer coat of the virus (capsid) with its corresponding receptor that gives a particular virus its characteristic tropism. For example, adenoviruses gain entry into respiratory epithelial cells by attachment of 1 of the 10 structural proteins with an integrin or immunoglobulin-like (Ig) receptor on the host cell surface, whereupon a second receptor (integrins \(\alpha_\beta\) and \(\alpha_\beta\)) binds with the penton base of the virus with further molecular events introducing the capsid to the endosome system for transport. \(^{10,32-34}\) The absorption form of viral entry is characterized by the presence of an outer envelope that is similar in structure to the host cell membrane, that is, coronavirus cold viruses (coronaviruses, SARS virus, rhinoviruses) \(^{28,35}\) and the myxoviruses (influenza, measles, respiratory syncytial, human metapneumovirus, and parainfluenza viruses) with respiratory cells. \(^{20,36,37}\) The similarity in lipoprotein structures of the two lead to fusion of the viral envelope with the cellular membrane/endosome system, and the virus is transported into the cell.

Once the virus gains access to the endosome system, a series of early molecular events (incubation period), some of which are still under the direction of the infective virion, uncoats the virus and releases the naked genome or nucleocapsid into the cell. \(^{8,9}\) The uncoating process may be the result of changing internal pH-ionic concentration or some other internal aspect of the host cell environment (lipids, proteins), virion-directed enzymatic activity, or multiple mechanisms in concert stimulating the unfolding of structural proteins away from the genome. At the same time, another set of coordinated events modifies the reproductive and macromolecular synthetic capabilities of the host cell to become a factory for replicating the next generation of viruses (replication). \(^{38-41}\) The way in which viruses store structural components in infected cells are released from infected cells or how their released components effect adjacent cells is what produces the characteristic changes in cells called cytopathic effects. \(^{23,42}\)

**Envelope**

The envelope is not a structural component of all viruses, but its presence more than ensures the carrier virion’s predilection for certain organs, tissues, or cells and adds protective layer(s) to the capsid. It is a lipid membrane derived from the host plasma membrane with the integration of glycosylated viral proteins. \(^{8,9}\) These proteins carry covalently linked oligosaccharide (poly-sugar) chains that are added posttranslation as the peptides are being transported to the cell membrane assembly point. These glycoproteins span the lipid bilayer by one or more
transmembrane segments, providing an anchor point for the molecule at the interior side and the characteristic knobs, projections, bonding sites, and so forth, of a specific viral group, for example, the HA, NA, and M2 proteins of the influenza A viruses. Envelopes are developed by one of two mechanisms (1) they are assembled internally and sequentially with subsequent budding from the host cell at maturation, and (2) the progeny and envelope are synthesized and assembled at the same time.

Type 1 envelope formation is a feature of influenza A viruses and is characterized by the replication and assembly of the ribonucleoprotein in the nucleus with subsequent M1 and NEP protein-dependent transport to the cytoplasm. At the same time, the viral glycoproteins (HA, NA) and the M2 membrane protein are synthesized and follow the cell secretory pathway and modify the outer host cell membrane. The M1 protein aligns the nucleocapsid and the inner layer of the modified membrane, and assembly is completed. In type 2 envelope formation, a feature of retroviruses, the virus is assembled around the MA segment of the Gag polyprotein bound to the inner surface of the plasma membrane. The Gag appears to direct the assembly process and the enfolding of the plasma membrane around the maturing viruses. Both pathways complete progeny development and prepare them for release to infect adjacent cells.

Genome

One of the most unique features of viruses is that they may contain either a DNA or an RNA genome. Every other replicating organism has both DNA and RNA, but DNA is the single source of genetic information for its reproduction. Moreover, the way and degree of competency with which progeny genomes are reproduced often govern the virulence and pathology characteristic of specific viral groups. Viral nucleic acids are defined as to whether or not they are single or double stranded, circular or linear, single or multiple copies, single or segmented, or ends are joined covalently or noncovalently. The sense of the viral polymerases is also considered an important characteristic of their respective genomes. Sense refers to the mechanism necessary for the genome to transcribe its genetic information to a functional messenger RNA (positive “+” strand mRNA). The complementing RNA and DNA strands would then be characterized as negative sense. This characteristic of strand complementarity relative to functional mRNA has been developed into a very effective schema for the molecular classification of viruses as shown below.

Recombination

The fact that there are a number of genotypes of the same virus as well as the emergence of new human viruses that share some fraction of their genome with similar animal viruses (avian influenza virus), provides de facto evidence that recombinant types of events can occur during viral replication. Some viruses exhibit rearrangements of nucleic acid sequences (genome copying errors, insertions, inversions, tandem repeats, reassortments), whereas others show that there has been recombination between two different genomes. Reassortment is common among segmented RNA viruses, such as the influenza virus, whereby two different segmented viruses infect the same cell and exchange RNA by exchanging segments during packaging. Because RNA viruses form many progeny with varying degrees of accurate template(s) replication, copying errors changing sequence information or exchanging of genetic information is not uncommon.

DNA viruses exhibit two forms of recombination, homologous and site specific. Site-specific recombination takes place along short DNA sequences flanked by codons recognized by catalytic recombination proteins and may occur with either or both nucleic acid strands. Homologous recombination occurs with all viral DNA, and it is an exchange between any pair of related sequences. These recombinant events are important in maintaining the virus in a changing environment, such as selection of a specific Influenza virus serotype or in perpetuating the virus without destroying the host as in the latent or persistent phase of the Herpetoviridae. Viral nucleic acids are defined as to whether or not they are single or double stranded, circular or linear, single or multiple copies, single or segmented, or ends are joined covalently or noncovalently. The sense of the viral polymerases is also considered an important characteristic of their respective genomes. Sense refers to the mechanism necessary for the genome to transcribe its genetic information to a functional messenger RNA (positive “+” strand mRNA). The complementing RNA and DNA strands would then be characterized as negative sense. This characteristic of strand complementarity relative to functional mRNA has been developed into a very effective schema for the molecular classification of viruses as shown below.

Viral Pathogenesis

Once gaining entry into the host, the virus must invade the host immune defense to establish the infection. This is accomplished by ligand attachment, followed by replication within the initial cell, and spread to contiguous cells. Once local infection is established, many viruses are spread further by one or more general disseminations via the lymphatics, reticuloendothelial cells, and/or the blood-
stream (viremia) to other tissues or cells with receptors that bind to the capsid epitopes. Viruses are capable of causing acute infections (influenza, common colds), persistent infections (cytomegalovirus, herpes simplex virus), or latent infections (Epstein-Barr virus, herpes simplex virus, varicella-zoster virus). Acute infections are characterized by a short incubation period, with a sudden explosion of symptoms (replication and release) that rise to a nadir and then descend as the host develops an adaptive immune response to the infection. Cellular damage is usually the result of a combination of release by cell lysis and the influx of host cytokines, antibodies, and immune-activated cells, which limit the spread of the virus and destroys infected cells. The cells and effectors of the adaptive immune response begin to remove the virus particles, and the attendant antibodies and memory cells produced during this process provide immunity to reinfection. Persistent infections are chronic and have some intervals between periods of waxing and waning. After the initial infection and limited viral release (or steady low level viral release without host cell damage), the adaptive response limits the infection and it appears quiescent only to appear later with repeated wax and wane cycles. Latent infections are the extreme end of the persistent infection spectrum—longer periods between overt signs of infection and adaptive control.

Adaptive immune clearance is accomplished by at least two mechanisms: (1) similarity of viral epitopes to host antigens and (2) genomic changes leading to changes in viral surface structure. In the first scenario, some viral epitopes are very similar to host cell surface epitopes, such that host immune clearance mechanisms do not recognize them as being non-self. These are usually viruses that attach near or are similar to antigens/receptors of the major histocompatibility complex (HLA, MHC) such that their epitopes create a condition of tolerance by downregulating MHC expression, neutralizing host immune response. The second adaptive mechanism involves the mutation frequency of the genome, extent of copying errors in replication, or recombination frequency of the genome. In the first scenario, small changes may occur in the structural peptides that do not interfere with the major mechanism of immune clearance, or small changes may occur in the viral structural peptides that slightly effect antibody or cytotoxic T-lymphocyte clearance, but there is some degree of protection (antigenic drift). These are usually due to random mutations and/or copy errors during replication. There are, however, instances when major changes occur in viral surface proteins, for example, when the capsid epitopes have changed so completely that the immune memory and clearance mechanisms directed against previous strains of the virus will not prevent infection (antigenic shift). These major changes in surface structure result from coinfection of two or more viral serotypes (same or different host origin) and subsequent recombination of their respective genomes.

The classic example of such an abrupt change or shift in viral surface structure with devastating virulence is the 1918 Spanish flu. The above-mentioned SARS and avian influenza strains are considered contemporary viruses with equal potential for similar lethal pandemics. It was only through a Herculean effort to isolate and quarantine infected humans, exposed family members and friends, exposed health care workers, and exposed and infected birds that the latter two viruses, for the present, have been localized to the Far East.

Thus far, there have been six major Influenza A antigen shifts since 1889. These long-term and discrete changes in antigenic structure teamed with the more common copying error–induced drift result in a diversity of immune memory within the population and serves to explain why some ethnic or age groups appear more or less protected by the current vaccine than others.

In summary, the type of pathology induced by a specific virus is a combination of several factors, including but not limited to its mode of attachment, where it attaches (the predilection for a specific organ, tissue), ease of cell-to-cell spread, its mode of replication, frequency of genetic change (extent of mutation, copy errors during replication, recombination), whether or not it destroys the host cell upon release or buds off the plasma membrane slowly or quickly, the presence of a characteristic pattern of cell destruction (cytopathic effect), and the point at which some form of immunomodulation occurs. The acute infection may, with its intense protein load spread over several cells, stimulate the production of antibodies, release cytokines, and attract cells that process and present viral antigens specific to various components, leading eventually to lytic release as observed in hepatitis virus infections. In other instances, there may be a downregulation of the T-cell cytolytic response to cells presented viral antigens as observed in latent herpesvirus infections or slow and persistent infections such as measles.

Finally, the virus may turn off some aspect of the immune system such as seen in the effect that human immunodeficiency virus has on T-helper cells at the time of attachment. Once attached the T-helper response is downregulated, having a cumulative effect over a long period of time, and may only be suspected when the ratios of CD4+ competent cells to CD8+ cells becomes inverted and the patient begins to exhibit a variety of opportunistic infections.

Classification

Traditional viral classification was based on a variety of physicochemical parameters reflecting the character and the geometry of the capsid as well as its size, structure, composition, organization, presence of an envelope, and
### Table 37.1. Classification of viruses.

| Family and viruses       | Gen (-)/BC; E; S*  | Receptors†              | Infection type‡         |
|--------------------------|---------------------|-------------------------|-------------------------|
|                          |                     |                         | Common Cold | Pharyngitis | Laryngitis | Sinusitis | Pneumonia |
| Orthomyxoviridae         | ssRNA (-)/V; E; H   | Cell surface sialyloligosaccharides | C           | C          | C          | C         |           |
| Influenza A virus        |                     | Cell surface sialyloligosaccharides | PU, AC      | C          | C          |           |           |
| Influenza B virus        |                     | α-2,6-Sialyloligosaccharides          |             |            |            |           |           |
| Avian influenza virus    |                     |                          |             |            |            |           |           |
| Paramyxoviridae          | ssRNA (-)/V; E; H   | Cell surface sialyloligosaccharides | C           | C          | C          |           |           |
| Parainfluenza virus types 1, 2, 3 | | Pattern recognition receptors: TLR4, CD14 | C           | C          | C          |           |           |
| Respiratory syncytial virus |                     | CD46, signaling lymphocyte activation molecule (SLAM, CD121) |             |            |            |           |           |
| Measles virus            |                     |                          |             |            |            |           |           |
| Metapneumovirus          | dsDNA/I; E; IC      | Coxsackie and adenovirus receptor (CAR) | C           | C          | C          | U         | U         |
| Adenoviridae             |                     | Major histocompatibility class II, CD46 | AC          | C          | C          | U         | C         |
| Adenovirus types 1, 2, 3 |                     |                          |             |            |            |           |           |
| (military)               |                     |                          |             |            |            |           |           |
| Coronaviridae            | ssRNA (+)/VI; E; H  | CEA glycoprotein family, aminopeptidase N, 9-O-acetylated sialic acid oligosaccharides | C           | C          | C          |           |           |
| Common coronavirus       |                     | Angiotensin-converting enzyme 2 | AC          |            |            |           |           |
| SARS virus               |                     |                          |             |            |            |           |           |
| Entroviridae (Picornaviridae) | ssRNA (+)/VI; IC   | Decay accelerating factor (DAF; CD55) | U           | U          | U          |           |           |
| Coxsackievirus           |                     | CAR, αvβ3-integrin (vironectin) |             |            |            |           |           |
| Echovirus                |                     | DAF (CD55), αvβ3-integrin | U           | C          | U          | U         | U         |
| Poliovirus               |                     | Poliovirus receptor | U           | C          | U          |           |           |
| Rhinovirus               |                     | Intracellular adhesion molecule 1 | C           | C          | C          | C         | U         |
| Bunyaviridae             | ssRNA (-)/V; E; H   | β3-integrin, upregulation RANTES | U           | U          | U          | U         | PU, AC    |
| Hantavirus§              |                     |                          |             |            |            |           |           |
| Herpesviridae            | dsDNA/I; E; IC      | Heparan sulfate, herpesvirus entry mediator (HVEM/HveA), nectin 1 (PRR1/HveC), nectin 2 (PRR2/HveB) | U           | C          | U          | U         | U         |
| Herpes simplex virus     |                     |                          |             |            |            |           |           |
| Cytomegalovirus          |                     | Heparan sulfate, epidermal growth factor | PC, AU      | U          | C          | U         | U         |
| Epstein-Barr virus       |                     | Complement receptor 2 (CD21) | C           |            | C          | U         | U         |
| Varicella-zoster virus   |                     | Insulin degrading enzyme, Fc receptor | PC, AU      | PC, AU     | U          | U         |           |
| Human herpesvirus 6      |                     | CD46                     | PC           | PU, AC     | U          |           |           |

*Gen (−), strandedness and nucleic acid type of genome (polymerase sense: + or −)/I–VI, Baltimore classification; ss, single strand; ds, double strand; E, envelope present; S, symmetry: IC, icosahedral, or H, helical.
†CEA, carcinoembryonic antigen; SARS, severe acute respiratory syndrome; TLR4, Toll-like receptor 4.
‡P, pediatric; A, adult; C, common; U, uncommon.
§Hantavirus: adult respiratory disease syndrome.
nucleic acid content. To take into account the unique aspects of replicating, transcribing, and translating viral genetic information, current classification schemes use a combination of viral physical characteristics and those of its genome (Table 37.1). The molecular approach, described by David Baltimore in 1971, makes the assumption that all viruses have to replicate to the point of creating a positive (+) sense RNA (functional mRNA) in order for the message to be translated by cellular ribosomes into the proteins necessary for the production and packaging of progeny viruses. This divides the currently known viruses into six functional groups, with groups II and III not represented by agents causing respiratory infections.

I. **Double-stranded DNA (dsDNA):** DNA separates into positive and negative strands, with the negative strand being translated to mRNA (adenoviruses, herpesviruses).

II. **Single-stranded DNA (+ssDNA):** A positive sense DNA strand replicates a −ssDNA intermediate that is then translated to mRNA.

III. **Double-stranded RNA (dsRNA):** The two strands separate, and the positive strand becomes the functional mRNA and the negative strand is translated to a functional mRNA.

IV. **Single-stranded RNA (+ssRNA), pathway (1):** The +ssRNA is translated to a −ssRNA replicative intermediate that is converted to a functional mRNA (picornaviruses/enteroviruses, togaviruses, coronaviruses).

V. **Single-stranded RNA (−ssRNA), pathway (2):** The −ssRNA directly replicates the functional mRNA (bunyaviruses, orthomyxoviruses, paramyxoviruses).

VI. **Single-stranded RNA (−ssRNA), pathway 3: The +ssRNA is translated to a −ssRNA replicative intermediate that is given rise to mRNA. This method of replication appears an exclusive characteristic of the Retroviridae. None of the known retroviruses causes overt respiratory pathology, but they do suppress the immune system and permit those viruses capable of respiratory disease to gain a foothold.

**Viral Respiratory Diseases**

**Orthomyxoviridae**

The orthomyxoviruses are ubiquitous enveloped viruses, approximately 90–120 nm in diameter with helical symmetry and segmented, negative sense, ssRNA genome. They are extremely stable in small droplet aerosols and are shed in large numbers, both characteristics adding in their efficient spread among immunologically naïve individuals and young schoolchildren. The most important member of the Orthomyxoviridae family is the influenza A virus, which reaches its peak infection rate in winter. Infections are described in terms of being local severe respiratory epidemic respiratory disease due to small changes in the peptide structure of the H and N peptidomers on the envelope surface (antigenic drift) or severe pandemic respiratory disease due to major structural shifts in these same molecules (antigenic drift). The salient points of their replication, structure, and antigen changes were described earlier in this chapter. Influenza viruses attach to mucosal surfaces, whereupon they replicate and spread in the respiratory tract leading to an acute, rapid-onset, febrile respiratory infection. There is a prodrome of fever, malaise, sore throat, and cough that progresses to croup, myositis, otitis media, abdominal pain, and vomiting or to viral pneumonia as the virus invades the central nervous system and muscles, as well as further damaging the lung parenchyma. The latter may be so severe as to cause hemorrhage, hyalination of the alveoli and alveolar ducts, and ulceration paving the way for secondary bacterial pneumonia.

Laboratory diagnosis is built around a number of quick serology tests based on the viral antigen and in latex agglutination, enzyme-linked immunosorbent assay (ELISA), or dipstick format. These viruses can be cultured, and confirmatory polymerase chain reaction (PCR) tests have been developed.

**Paramyxoviridae**

Paramyxoviruses are enveloped viruses with helical symmetry and are 150–300 nm in diameter with a nonsegmented negative sense ssRNA genome. This viral group runs the gamut of respiratory symptoms from severe respiratory syncytial virus infections in infants (common), to the moderate to severe parainfluenza virus (common) and metapneumovirus (uncommon) infections, to the common measles (rubeola) virus with its mild respiratory prodrome and accompanying rash, its most identifying characteristic.

The parainfluenza viruses have four distinct HN fibers attached to their outer envelope, dividing the group into four distinct types. Parainfluenza type 3 virus is the most common type isolated, especially in children ≤6 months of age, and endemic peak infectivity occurs in late spring. Type 1 viruses are isolated about half as frequently as type 3 and type 2 about half as frequently type 1. Both types 1 and 2 appear seasonally, with their peak isolation period occurring in the summer and alternate years. Infections range from mild upper respiratory infections (common cold-like syndromes) to severe infections of the large airways of the lower respiratory tract (croup, laryngotracheobronchitis).

The HN fibers serve as a ligand to sialylated molecules on the cell surface of ciliated respiratory epithelial cells.
Anchoring the virus to the cell surface activates the F (fusion) protein, which in turn is cleaved by cell surface serine proteases, and the virus enters the host cell. Upon entry, the nucleocapsid complex that consists of the NP (nucleocapsid), L (polymerase), and P (phosphorylated nucleocapsid-associated protein) is released along with ssRNA. These proteins and accompanying viral genome serve to direct mRNA synthesis. The mRNA replicates an antisense strand for progeny genomes and is also translated to form the necessary proteins for new virus production. Viral assembly occurs in the cytoplasm, with the new NP proteins condensing around the newly replicated genomes forming a helical structure that complex with the P and L proteins to form a complete nucleocapsid.

Envelope proteins have been simultaneously transported to the cell surface by the secretory endoplasmic reticulum, and the entire complex is assembled at the apical position of the host cell and released by budding. This apical release is into the mucin layer, preventing infection of the deeper cell layers, and the presence of the fusion protein results in some syncytia formation. Laboratory diagnosis is most commonly by cell culture and antigen–antibody detection, with some successful molecular techniques having been reported.

The most common cause of severe lower respiratory disease in children is respiratory syncytial virus. These viruses are typical paramyxoviruses and are similar to the parainfluenza viruses in that they are ubiquitous, are 120–300 nm in diameter, and have a ssRNA, negative sense, nonsegmented genome with a fusion glycoprotein (F protein) on the envelope surface. The envelope is highly pleomorphic and is circumscribed with glycoprotein projections that, in contrast to the parainfluenza viruses, consist of three transmembrane glycoproteins: the aforementioned F protein as well as the G and SH proteins. Infected cells become infected, more viruses are released, and a secondary viremia occurs, leading to the characteristic rash formation of the infection.

Most individuals exhibit evidence of infection by the end of early childhood, with severe disease requiring hospitalization occurring in children ≤24 months, peaking in children ≤6 months old. Infections are seasonal, with onset in winter to early spring. Severity of disease is age related, as adults exhibit mild cold-like symptoms with or without rhinitis, children exhibit mild disease to pneumonia (inversely proportional to age), and infants exhibit severe seasonal pneumonia, bronchiolitis, and tracheobronchitis. The severe bronchial disease seen in infants is probably due to a combination of narrow airways and swelling tracheal and bronchial tissue due to viral-induced pathology and host immune response. The economic impact of this seasonal infection, because of the virus’ highly contagious nature and virulence, has led to recommending administering prophylactic immune globulin to prevent epidemic infection in susceptible populations.

These viruses grow in culture in HEp2 cells, HeLa cells, and cells adapted from a type II human alveolar epithelial lung carcinoma (A549). However, because of the extreme lability of the virus in clinical specimens, culture is not the most commonly used laboratory diagnostic procedure. Routine laboratory diagnosis is made through a variety of rapid direct or indirect antigen and/or antibody tests in either the immunofluorescence or ELISA format. Some recent reports have shown that molecular tests, especially reverse transcriptase PCR (RT-PCR) may also be effective in the rapid diagnosis of this infection.

The rubella virus (measles virus), also a paramyxovirus, is ubiquitous and highly contagious in an immune naïve population. Infections are transmitted by inhalation of large droplet aerosols, with the peak infection period occurring from autumn to spring. Their structure is typical of the family, consisting of an outer envelope (100–250 nm diameter) surrounding the helical NPL nucleocapsid complex. The outer envelope exhibits two types of peplomeric projections, a conical-shaped hemagglutinin (H glycoprotein) and a dumbbell-shaped fusion (F glycoprotein), and a neuraminidase (NA) protein has also been described. As with the other paramyxoviruses, these peplomers are involved with attachment and fusion of the virus with the host cell. Attachment and virus entry occurs via the F protein liganding with CD46 or a signaling lymphocyte activation molecule (SLAM, CD121/Cdw150) on mucosal epithelial surfaces. Once the virus enters the cell and the genome is uncoated, replication is typical for the nonsegmented paramyxovirus ssRNA genome.

Adenoviruses are infected through membrane release and syncytium formation, eventually giving rise to a primary viremia, and further spread is by blood-borne cells of the reticuloendothelial system. Multiple organs become infected, more viruses are released, and a secondary viremia occurs, leading to the characteristic rash and Koplik’s spots associated with the disease. The primary viremia also gives rise to the upper respiratory prodrome that is present prior to the rash’s appearance, and symptoms range from mild to severe in nature and with or without a cough. In severe cases, respiratory symptoms may be augmented by the secondary viremia, which affects the entire respiratory mucosa, including denuding ciliated cells from the mucosal surfaces, and compromises the patient further through cough, croup, bronchiolitis, and pneumonia.
Diagnosis is primarily clinical, based on cough, coryza, rash, and Koplik’s spots. The most common laboratory test is a fourfold specific antibody titer movement using a number of techniques, including complement fixation, ELISA, immunofluorescence, and, to a lesser extent, neutralization.69 There are also several antigen detection tests available in a variety of formats for detecting rubella antigen from serum, nasal discharge, and urinary sediments, and an RT-PCR test for measles RNA has been described.69,81

The recently described human metapneumovirus appears also to be a ubiquitous pathogen of children like respiratory syncytial virus and exhibits a close genetic association with avian pneumoviruses.48,82 Whereas Respiratory syncytial virus tends to be severe in infants, with most children exhibiting seroconversion of infection by age 2 years, only 50% of children have metapneumovirus antibodies by 2 years, eventually reaching 100% seroreversion by age 5.83–85 They are typical paramyxoviruses with a negative sense, ssRNA, helical nucleocapsid, and a pleomorphic outer lipid envelope with peplomers projecting from the surface.86 These surface projections are the F (fusion) protein, which permit viruses to enter host cells, release progeny, and infect adjacent cells, leading to characteristic syncytia. The actual receptor molecule is unclear but appears to be associated with Toll-like receptor 4.8,86 Major symptoms include nasal congestion, cough, fever, and rhinorrhea, and the peak periods of infection appear to coincide with influenza and respiratory syncytial virus.84,85 Diagnostic tests include serology (enzyme immunoassay, indirect fluorescent antibody), viral culture, and RT-PCR.86–88

Adenoviruses

Adenoviruses are nonenveloped, 70–90 nm diameter, icosahedral, double-stranded DNA viruses.8,9,61 Their clinical importance resides in the ability to cause acute respiratory and conjunctival infections, diarrhea, central nervous system disease in humans, as well as latent or persistent infections in certain animals and cell lines.8,9,32,61 They have been shown in two large epidemiologic studies to be the most commonly isolated virus in either clinical or subclinical infections.61 Their typical cytopathic effect in susceptible cells consists of large rounded cells with fibrils or strands attaching them together.42 They gained their name in the early 1950s as a result of their association with spontaneous degeneration of explanted adenoid tissue.61 There are currently 52 serotypes as defined by their capsid proteins, and about half these serotypes are considered the etiologies of specific human diseases.61 Group- and type-specific immunologic identities are conferred by hexon, penton, and fiber capsid proteins. Viruses enter the cell by attachment of knob-capped spikes or fibers projecting from the base of penton capsomeres with the coxsackie adenovirus receptor and subsequent endocytosis.19,21,32,33,89 This is facilitated by the association of the penton base with a cellular integrin and the partial uncoating of the virion. Upon entering the cell and further uncoating, the genome is transported to the nucleus; mRNAs are formed and then exported to the cytoplasm for transcription of progeny proteins.8,38,65 Viruses are then assembled through a series of steps that neutralize host defense mechanisms, cleaving of precursor proteins, and release of cell. Infections may be lytic, persistent or chronic, or oncogenic depending on the host cell type or source (humans, animals, cell cultures).89

Respiratory-associated adenoviruses in children and adults consist of a few specific serotypes including members of subgenus B,46,85 subgenus C,18,34,51,70 and serotype 4, the only member of subgenus E.61,90 These viruses have been shown to be latent in lymphoepithelial tissue, nasopharynx, and other tissues. Adenoviruses 1, 2, and 5 have been isolated from infants with pharyngitis and coryza or who have otherwise been asymptomatic, and children exhibit a wide variety of clinically apparent respiratory syndromes associated with tonsils and adenoids,18,34,51,70 upper respiratory disease,8,38,34,51,61,70 intussusception,18,51,61,70 and pharyngoconjunctival fever.46,83 Serotypes 3, 4, and 7 are common etiologies of acute viral respiratory disease in young adults, with serotype 4 often associated with epidemic infections in closed populations such as military recruits and types 5, 31, and 34 responsible for viral pneumonia with concomitant dissemination in immunocompromised patients.

Depending on the syndrome, adenoviruses may be cultured from pharynx, sputum, conjunctival scrapings, urine, and stool. However, two large and independent studies of symptomatic and asymptomatic individuals demonstrated that adenoviruses are more commonly isolated from stools than from respiratory or other clinical specimens.51 They are readily cultured in human epithelial cells with characteristic cytopathic effects visible within 2–7 days.42 They may also be visualized in purified preparations or in direct specimens by electron microscopy. Their DNA can be detected in cultures or clinical specimens by amplification or amplification probe methods with appropriate primers.71 The standard method of laboratory diagnosis still remains the detection of a fourfold movement in antibody or antigen titer by a variety of serologic techniques, including complement fixation, neutralization, hemagglutination, immunofluorescence, and ELISA.8,61

Coronaviruses

Coronaviruses are highly pleomorphic viruses named for the “corona-like” array of club-shaped surface glycoproteins (S proteins) that project from their surface.3,8 These viruses are ubiquitous and have been traditionally
considered major pathogens of the upper respiratory tract in humans, causing approximately 15% of upper respiratory infections reported in temperate climates. They occasionally cause viral diarrhea and are also considered a major animal pathogen, likely composed of three distinct antigenic groups. Coronavirus are enveloped, 80–160 nm diameter helical viruses with an infectious, single stranded, polyadenylated positive sense RNA genome. The genome is the largest known viral RNA and forms a unique mRNA that has a 3′ polyadenylated cap that forces transcription to occur from the 5′ direction. They gain entry by attachment of S-protein projections or rays to aminopeptidase M or 9-O-acetylated sialic acid containing oligosaccharides on the surface of nasal epithelial cells but do not produce characteristic cytopathic effects. Once attached, viruses enter the cell by pinocytosis, and all progeny synthesis occurs in the cytoplasm. The positive sense genome is released to form a negative sense intermediate; the resultant positive mRNAs nest at their 3′ polyadenylated ends and translation occurs at the 5′ ends. The mRNAs are translated into nonstructural proteins and a variety of structural and biosynthetic proteins, including RNA-RNA polymerase, ATP-helicase, surface hemagglutinin-esterase (HE) protein (some coronaviruses), small envelope protein (E), membrane glycoprotein (M), and the nucleocapsid protein (N). Viruses are assembled in the cytoplasm and then bud into vesicles from the endoplasmic reticulum and are released from the cell membrane by reverse pinocytosis or by lysis, destroying the host cell. Changes in envelope or capsid antigenic structure, as described above, may result in immunologically different peptides being formed, making it difficult to develop consistent serodiagnostic reagents or vaccines for this group. Because of the difficulty in growing coronavirus strains in culture, the benign and self-limiting nature of the disease (common cold), and the difficulty in obtaining definitive reagents for the laboratory, diagnosis is clinical and treatment is palliative.

Severe Adult Respiratory Distress Syndrome

The recently described SARS coronavirus (SARS-CoV), first identified in China’s Guangdong Province, has been identified as the etiology of an acute, severe, and often fatal lower respiratory and systemic disease (SARS), characterized by a severe atypical pneumonia. Although morphologically consistent with common coronaviruses, the SARS virus is genetically quite different from its human and animal counterparts, appearing to comprise a fourth antigenic group evolutionarily equidistant from the three major groups of coronaviruses. This may reflect a closer relationship to the animal virus from which it was derived. The SARS virus lacks the HE protein and appears to attach to host cells by 9-O-acetylated sialic acid containing oligosaccharides, and it may be cultured in VERO cell lines, where it may produce a syncytium. Its ease in culturing has permitted the development of some very sophisticated molecular approaches to strain identification, such as real-time PCR and sequence-based typing.

Bunyaviridae (Hantaviruses)

The hantaviruses are ubiquitous and are associated with adult respiratory distress syndrome (Hantaan virus) and pulmonary syndrome, shock, and pulmonary edema (Sin Nombre virus). Viruses are inhaled from aerosolized rodent urine with each virus species apparently adapted to its own specific rodent vector. These are enveloped RNA viruses about 90–110 nm in diameter with helical symmetry and contain three negative sense ssRNA segments. The genome is referred to as “ambisense” because not only can it be translated into a functional messenger but also the negative strand encodes information for six proteins, some nonstructural proteins, viral RNA−RNA polymerase, and the G1 and G2 proteins. The latter are associated with cell fusion and hemagglutination and act as a receptor for neutralizing antibodies. The virus attaches to respiratory mucosal surface β3-integrin molecules, and replication is cytoplasmic with subsequent release by budding via the Golgi/endoplasmic reticulum/vesicle pathway. The Sin Nombre virus differs in that it is assembled at the cell cytoplasmic membrane and then is released by budding. Symptoms include acute onset fever and malaise, indicative of localized cellular damage and viremia leading to increased lung vascular permeability and shock. A secondary viremia results in spread to target organs as well as increased vascular endothelial damage and invasion of macrophages. Within 4–5 days, there are generalized respiratory symptoms (dyspnea, cough, hypotensive, malaise) that, if untreated, can abruptly accelerate to acute pulmonary failure, pulmonary edema, renal failure, and shock. The characteristic histopathologic picture is that of alveolar edema accompanied by nonnecrotic, interstitial T lymphocytic infiltrates and few to no visible polymorphonuclear leukocytes. Laboratory diagnosis is by ELISA serology for the detection of specific IgM (acute disease) and/or IgG titers or antigen, RT-PCR of blood or other specimens, and cell culture. The latter is discouraged in all but designated public health laboratories because of the extremely infectious nature of the virus.

Picornaviruses

The picornaviruses are a large heterogenous group of small (20–30 nm), nonenveloped, icosahedral viruses that include some of the enteroviruses: (1) coxsackie A with 24 antigen types, (2) echovirus with 33 types, (3) poliovirus with 3 types, and (4) rhinovirus with over 100 types.
These are ubiquitous, positive sense ssRNA viruses and attach to host cells by a variety of receptors that give each virus species its characteristic tropism (see Table 37.1). Transmission is either by the fecal–oral route (enteroviruses) or by aerosol and contaminated hands (rhinoviruses). Poliovirus has been nearly eradicated on a global basis and infection is sporadic, enteroviral infections are more common in summer, and rhinovirus infections appear in early autumn and late spring. The mucosal surface of the oropharynx serves as the primary portal of infection for picornaviruses, with enteroviruses also having the gastrointestinal tract as an additional port of entry. These cells have a variety of receptors that can accommodate the different capsid proteins of members of this group (see Table 37.1). Rhinovirus infections are thought to be limited to the upper respiratory tract and are associated with common cold-like infections and exacerbations of asthma, although there are some indications that they can cause lower respiratory infections as well. Enteroviruses exhibit a wide variety of secondary targets and host age depending on the species. For example, enterovirus infections are characterized by a serious central nervous system component (paralysis, encephalitis, meningitis); coxsackie A and B viruses and echoviruses are also associated with carditis, rash, and serious infections in newborns and neonates; whereas poliovirus infections occur more often in young children. Laboratory diagnosis of picornaviruses is by serologic tests for antibody and antigen and by PCR.

Herpetoviridae

The herpesviruses, in general, are minor viral respiratory pathogens. It is only when an infection occurs during the peak season of one of the more common respiratory pathogens do they have to be considered in a differential diagnosis. The human Herpetoviridae are large (150–200 nm), enveloped, icosahedral dsDNA viruses that undergo recombination with the host cell genome to establish a latent (asymptomatic) relationship with the host. Infections may be primary only or progress to dissemination and, in either case, may result in a latent infection. Primary infections occur through exchange of saliva or other bodily fluids and subsequent viral attachment to mucosal surfaces via specific receptors for each member of the group, and initial replication of the virus occurs through a double-stranded linear DNA that leads directly to a positive mRNA. After progeny release, there is adjacent cell infection and eventual cell-to-cell spread from mucosal epithelium to target cells, organs, or tissues with a latent state set up in neurons or reticuloendothelial cells. During the initial replication or during the viremic state, viruses may be transported via lymph nodes, macrophages, or B cells to various extramucosal tissues or organs. The respiratory component of these infections consists of a viral prodrome of headache, fever, malaise, rhinitis, and/or pharyngitis due to the release of various cytokines. The following are the major herpesviruses and their clinical manifestations:

1. **Herpes simplex virus**: Virus attaches to the mucosal surface by nectin or heparin sulfate. After progeny release, there is adjacent cell infection and eventual cell-to-cell spread from mucosal epithelium to the sensory and autonomic ganglia of the peripheral nervous system, where a latent infection is established. Primary replicated virus may also be transported by the bloodstream to other organ systems and then establish latency in the sensory and autonomic ganglia. Reactivation of these latent peripheral nerve viruses or those from infected organs may also directly infect the brain and spinal cord. Diagnosis is made by antigen/antibody tests to respective types, a polyclonal test for both in fluorescence or EIA format, and molecular tests.

2. **Cytomegalovirus**: Viruses attach to the host mucosal cells by heparin sulfate or epidermal growth factor and then spreads to lymph nodes, where T cells and macrophages are infected. T-cell infection leads to a mononucleosis-type presentation, and macrophage infection leads to multiorgan dissemination and cytomegalic inclusion disease. Cytomegalovirus usually causes subclinical infections in immunocompetent individuals, with more devastating infections occurring among the immunosuppressed (e.g., hematology and oncology patients and allograft recipients). Diagnosis is made by antigen/antibody serology (fluorescence and ELISA) and molecular tests.

3. **Epstein-Barr virus**: Epstein-Barr virus infects the oral mucosa and B cells by attaching to complement receptor 2 (CD21). The B cells are transformed (immortalized) and transport the virus to the liver (hepatitis), tracheobronchial tree (pharyngitis), or spleen. In cases of mononucleosis, the spleen is highly enlarged and exhibits atypical lymphocytes. Epstein-Barr virus is also associated with lymphomas in the immunosuppressed, Burkitt’s lymphoma, and nasopharyngeal carcinoma. Diagnosis is made by antigen/antibody serology (fluorescence and ELISA) and molecular tests.

4. **Varicella-zoster virus**: Using the insulin-degrading enzyme or Fc receptor as a point of attachment, varicella-zoster virus infects epithelial cells and fibroblasts. Viruses spread from mucosa to lymph nodes with subsequent transport to liver, spleen, and respiratory systems. It then spreads by viremia to the skin, where it causes the characteristic skin lesions (chickenpox). The virus eventually establishes a latent infection in the sensory ganglia, with later reactivation possible (shingles). Other than the clinical presentation, serology is the
primary means of diagnosis; a few molecular alternatives have been described.50

5. Human herpesvirus type 6 (roseola)102,112–114: Formerly called human B-cell lymphotrophic virus, human herpesvirus type 6 is a ubiquitous and typical herpesvirus in structure and replicative mechanism. It infects most children before they reach 2 years of age, with specific antibodies found in 64%–83% of children within their first 13 months of life. Viruses attach to CD4+ cells (T cells, monocytes, macrophages, etc.) by a CD46 ligand and, in fact, upregulate CD4+ in CD4+ cells. Primary infection appears to occur by droplet/body fluid aerosols reaching the oropharynx. From the oral mucosa, the virus spreads to the regional lymphatics and then to mononuclear cells. Lymphocytes are the main carrier during primary infection but the virus persists in monocytes and macrophages. The infection expresses itself with a high fever of moderate duration (3–5 days), with accompanying mild upper respiratory symptoms and cervical lymphadenopathy, and, as the fever resolves, a classic maculopapular rash appears (exanthem subitum, sixth disease). Laboratory diagnosis is by fourfold titer movement of specific antibody or by molecular techniques.102,113,115,116

Conclusion

We have seen that respiratory manifestations of viral infections may be only a minor harbinger of more serious systemic disease, or viral invasion, in and of itself, may have the potential for causing lethal pandemic respiratory infections.31,108 Regardless of the degree of initial respiratory compromise, viral respiratory diseases are the cause of more human morbidity, mortality, and health care spending than all other infectious diseases. What makes controlling these viruses an almost insurmountable task is the frequency of mistakes in replication or in recombination that give rise to variants for which there is no immune memory in the population, and hence no immune protection, or to variants that are resistant to current antiviral therapies.65 As the human population increases, the average age of the population increases with its parallel decrease in immune function and increase in the number of immunosuppressed individuals or well-maintained terminally ill individuals; therefore, the chance for one of these mutant viruses or one that has adapted to humans directly from a natural reservoir increases geometrically.75,106,117

Because of the proven devastating potential of viral respiratory infections, the one common thread has been the search for more rapid and more accurate means of diagnosing these infections and identifying their etiologic agents.42 These lines of endeavor are mandatory for two primary reasons: (1) the lack of vaccines specific enough to protect against reinfection or against all variants within a virus taxon and (2) the very narrow window for effective antiviral therapy during early infection.118 Understanding the molecular biology of the respiratory viruses has helped to address this “need for speed” by directing the development of rapid molecular diagnostic techniques such as the application of specific probes for virus identification in tissue slides or specimen smears (fluorescence in situ hybridization, PCR) or in fluids and cells (real-time PCR, RT-PCR, sequence-specific oligonucleotide probes, multiplex PCR techniques).41,70,72,106,109,111,116,119,120 Molecular tests will enable pathology departments and laboratories to offer rapid identification of a specific infectious agent when several cause the same set of symptoms, permitting more accurate therapy to be more effectively administered and, ultimately, decreasing patient stays and health care costs.8,115 To accomplish this, however, there must be a dramatic increase in funding for research into the genetics of these viruses, the development of recombinant polyvalent vaccines, the development of public health care strategies to control pandemics, and the development and bringing to market of rapid, accurate, and specific diagnostic tests.

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