Features of respiratory syncytial virus

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Abstract

Respiratory syncytial virus infects almost all of us in early childhood and causes most cases of bronchiolitis – the most common reason to be admitted to hospital under 1 year of age in the developed world. Each winter, paediatric wards are tested by predictable epidemics of unpredictable intensity. Trials of vaccination in the 1960s were associated with catastrophic enhanced illness leading to intense research that has enriched immunology and virology as a whole. Treatment is still supportive but with modern facilities and training survival rates are high even in severe disease. Post bronchiolitis wheeze is common and hard to treat but new therapies are being proposed. Possible links between severe bronchiolitis atopy and asthma are still under investigation.

Keywords allergy; bronchiolitis; cell mediated immunity; lymphocyte; respiratory syneytial virus

History

Outbreaks of feverish respiratory illnesses have been described for many centuries and the word 'influence' (from which 'influenza' comes) was used from the 14th century to imply that the disease was affected by astrological events. Goodpasture published the first account of pneumonia in infants caused by epidemic virus infection in 1939 and Adams was probably the first to give a detailed description of an outbreak of RSV in 1941. He described a nosocomial infection in a newborn nursery that affected 32 children causing nine deaths and found cytoplasmic inclusions in the lung at post mortem. The virus itself was first isolated from a chimpanzee with a coryzal illness in 1956. The virus was cultured and shown to be able to infect other chimpanzees and was named 'chimpanzee coryza agent'. In the following year, Chanock and others isolated the same virus from a child with croup in Baltimore. The virus was renamed respiratory syncytial virus (RSV) to describe the site of infection and characteristic syncytium formation found in cell culture and infected tissues.

Epidemiology

RSV is distributed worldwide with similar patterns of disease in all climates. Wherever it is found, children are the ones most likely to have the severe illnesses. Bronchiolitis is the single commonest reason for a child under 1 year of age to be admitted to hospital in the developed world. Re-infections occur throughout life, in spite of good levels of neutralising serum antibody. As well as the very young, the very old are also at risk from RSV infection. Outbreaks of RSV pneumonia in the elderly are possibly as important as influenza in causing excess deaths. Annual outbreaks occur that are usually of sharp onset and follow regular predictable patterns. In temperate climates most cases of RSV infection are reported during the winter months. Most hospital admissions occur during a narrow peak period lasting only a few weeks. In the UK, the peak incidence is between the beginning of January and the end of March. In tropical climates such as in Hong Kong and Trinidad, epidemics are seen during the rainy season. Influenza epidemics also occur in the winter but do not usually coincide with the peak of the annual epidemic of RSV infection. Clinical isolates of RSV are rare in the UK during the summer months and it is not clear where the virus goes or how it re-emerges so rapidly during the next winter season. No animal reservoir for human RSV is known to exist.

Initial studies of strain variation in RSV came from monoclonal antibody typing into A and B strains. Both strains commonly co-circulate in one outbreak. There is little difference in the type or severity in clinical disease produced by A or B strains. The possibility still exists, however, that some strains, not defined by monoclonal studies, may cause more severe disease. Molecular biological techniques have allowed a more detailed study of subtype variation within RSV, by classification according to genome sequences especially of the highly variable G protein gene. Interestingly, viruses sequenced from children all over the world fall into the similar lineage classifications although the relative frequency of each lineage differs. Unlike influenza virus, several strains of RSV commonly circulate locally alongside one another with similar frequencies. Some evidence of evolution of RSV strains has emerged, some strains becoming less common over the years and others increasing in frequency. This may be occurring through immune selective pressure and the common ancestor of the current B strains has been estimated to date back to around 1949. The divergence between A and B strains has been calculated to have occurred approximately 350 years ago.[1]

Taxonomy: the mononegavirales

RSV belongs to the order mononegavirales, which consists of viruses with non-segmented negative-stranded RNA genomes. In this group, the entire virus is coded within a short length of fragile RNA written in a negative sense and which therefore needs first to be transcribed within the infected cell into positive sense mRNAs to allow protein transcription.

The order includes three families of virus: the filoviridae, paramyxoviridae and rhabdoviridae. All have similar genomic organisation and patterns of morphogenesis and are differentiated by their biological differences.[2] Paramyxoviridae, including RSV, are transmitted by contact or aerosols and isolated from warm-blooded vertebrates suggesting the family evolved relatively recently. They are generally associated with respiratory illnesses. Filoviruses have only been isolated in sporadic cases or outbreaks, and cause severe haemorrhagic illnesses in primates and man. Rhabdoviruses are widespread and infect both mammals and other organisms even some plants. They are mostly spread by arthropod vectors and, with the exception of rabies, cause only mild illness in humans.

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Paramyxoviridae (or paramyxoviruses) are divided into two subfamilies: paramyxovirinae, and the pneumovirinae, to which RSV (genus pneumovirus) belongs. In recent years, a second human pneumovirus, human metapneumovirus, has been discovered and found to be an important human pathogen especially in children. The paramyxovirinae can be further subdivided into three genera: rubulavirus (e.g. mumps), morbillivirus (e.g. measles) and paramyxovirus also known as parainfluenzavirus.

Mononegavirales share a pattern of 5–10 genes arranged linearly with the viral polymerase (L) at the 5′ terminus. Some 95% of the gene sequence codes for polypeptides. Comparison of gene sequences from several paramyxoviruses, rhabdoviruses and the filoviruses Ebola and Marburg suggested that pneumoviruses were indeed a separate lineage. Intriguingly the L (polymerase) and N (nucleoprotein) of RSV has closer homology to Ebola and Marburg than to the other pneumoviruses.[3,4]

Molecular biology

RSV virions are heterogeneous in size and shape with pleomorphic spherical or filamentous forms. Spherical particles are approximately 60–100 nm in diameter. The nucleocapsid contains the single strand of non-segmented negative sense RNA of about 15222 nucleotides in length.[5] Virions assemble at the plasma membrane of an infected cell, are released by budding and take a lipid bilayer membrane derived from the host cell. Infectious RSV may well be in a filamentous form like the filoviruses (Ebola and others). The envelope of the virus is covered by projecting spikes of 11–20 nm separated by 6–10 nm. These are made up of the three surface glycoproteins – G, F and SH.

Proteins of RSV

RSV is tiny, neat and beautifully put together. It consists of only 10 coded proteins. Three are surface transmembrane proteins: G, F and SH (small hydrophobic). Two are non-glycosylated matrix proteins: M and M2. Three are associated with the viral genome and make up a nucleocapsid: N, P and L. Two further proteins are described as NS1 and NS2 (non-structural). RSV protein sequence diversity has been studied and it appears that the surface protein G is highly divergent with only about 50% amino acid sequence homology between strains and the internal proteins, such as N, highly conserved with greater than 90% homology.

The F (fusion) protein is closely related in structure and function to surface proteins found in other paramyxoviruses. It is involved in virus penetration into cells and syncytium formation. Efficient syncytium formation also requires the presence of the other surface proteins, G and SH.[5]

The G protein mediates attachment. It is highly serine threonine rich (31%) and extensively *O* glycosylated, an unusual feature in viral proteins. *O*-linked glycosylation is not found in any other paramyxovirus, orthomyxovirus or rhabdovirus. It is, however, described for the G protein of filoviruses again suggesting a possible common origin.

SH is a small hydrophobic protein of 64 amino acids. It has been suggested that as well as being important in syncytium formation it may play a role in blocking cell death through apoptosis and inhibition of TNF-α signalling.

Infectious cycle

In vivo

Transmission of RSV is by introduction of infected secretions onto the mucosa of the eyes or nose. Usually this is by self-inoculation with the hands after touching infected secretions or contact with virus on objects such as doors, surfaces or fomites. Aerosolisation seems to be a less frequent means of spread.[6] Infection through the eye alone has been shown to be sufficient to cause respiratory illness.

The virus is capable of remaining infectious for several hours on inanimate objects given optimal conditions. In hospitals this means so many items including stethoscopes, door handles, lift call buttons, computer keyboards, toys and telephones provide opportunities for nosocomial spread.

RSV infects the upper respiratory mucosa of the nose and the conjunctivae but not buccal and oral mucosa. The mechanism of spread to the lower respiratory tract is not known, but assumed to be via the respiratory epithelium or aspiration of infected secretions. The incubation period between inoculation and disease is about 4–5 days from studies in adults. Lower respiratory tract signs appear 1–3 days after the onset of rhinorrhoea. In post mortem studies, using immunofluorescence, RSV is only found in the superficial epithelial layer.

Viral antigens have been reported to be present on circulating mononuclear cells from some individuals. With the use of reverse transcriptase polymerase chain reaction, viral RNA has been shown to be present in cells in circulating blood in bronchiolitis suggesting a mechanism by which RSV might spread outside the respiratory tract.

Virus shedding is often for more than 2 weeks, after the peak of the illness during resolution. RSV can be detected by conventional methods even 4 weeks after the onset of bronchiolitis in immunocompetent children.

In vitro

RSV grows best in cultures of human cells such as HEp-2 and HeLa cells. In culture, it is associated with a characteristic effect of syncytium formation with eosinophilic cytoplasmic inclusions. RSV is relatively labile: it is destroyed rapidly at 55°C, it does not tolerate freeze-thawing well and can be inactivated by ultraviolet light.

It is not known which cellular protein provides the site of attachment for RSV. However, *in vitro,* the cellular receptor or receptors appear to be very abundant on the cell surface. Cell penetration is by fusion and the viral envelope may become incorporated into the cell surface. After this, all the events of replication occur in the cytoplasm independent of the nucleus and with little effect on other cellular functions. The RSV genes are transcribed in their 3′ to 5′ order into a positive strand RNA by the transcriptase L from which the proteins are synthesised. Viral mRNAs and proteins can be detected 4–6 hours after infection.

Virion assembly occurs at the plasma membrane. Nucleocapsids preformed in the cytoplasm collect near plasma membrane sections containing concentrations of transmembrane RSV glycoproteins and virion formation occurs by budding. Budding occurs from apical surfaces in well-circumscribed cell surface regions perhaps suggesting a dependence on local host sub-membranous structures.