**Respiratory syncytial virus**

Part I: From genome to proteome analysis

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1.1. Taxonomy and structure

1.2. Respiratory syncytial virus (RSV) was recovered in 1957 and was quickly recognized as a common and important cause of respiratory tract infection in infants and young children (1,2,3). It belongs to the order Mononegavirales, family Paramyxoviridae, subfamily Pneumovirinae, genus Pneumovirus, species Human respiratory syncytial virus (4). RSV is so named because its replication leads to the fusion of neighboring cells into large multinucleated syncytia. The virus, 150 to 300 nm in diameter, has a single-stranded negative sense RNA (15.2 kb) with ten genes that are transcribed sequentially in the 3' to 5' order; genome codes for 11 virus-specific proteins (5,6). Virus particle comprises a ribonucleoprotein core formed by interaction between the viral genomic RNA (vRNA), the nucleocapsid (N) protein (391 amino acids, 42 kd), the phospho (P) protein (241 amino acids, 35 kd), and the large (L) protein (2,165 amino acids, 250 kd), which is the catalytic component of the replicase-transcriptase complex and possesses RNA-dependent RNA polymerase activity (7) (Figure-1).

![Figure 1. Diagram of RSV particle](image)

The ribonucleoprotein core is visualized by electron microscopy as a strand of repeating N protein subunits that tightly bind along the entire length of genomic RNA and form a herringbone-like structure of 10–20 nm in diameter. Proteins M2-1 (factor essential for viral viability, 194 amino acids, 22 kd,) and M2-2 (90 amino acids, 28 kd) regulate the activity of the polymerase (8-10). Viral lipid envelope bears three proteins: G glycoprotein (282-319 amino acids,90 kd), by which the virus attaches to cells; F fusion (70 kd) glycoprotein, which facilitates entry of the virus into the cell by fusing host and viral membranes (F0-precursor activated by cleavage into disulfide-linked F1 (48 kd) and F2 (26 kd) subunits); and the small hydrophobic SH (64 amino acids) partially glycosylated protein, which is important for viral infectivity and is a potential viroporin (11-14). M (256 amino acids) nonglycosylated protein present in the inner viral membrane is essential for forming a virus particle due to its interaction with F protein during virion morphogenesis, and traffic between the cytoplasm and the nucleus (15,16). Additionally, two non-structural proteins, NS1 (139 amino acids) and NS2 (124 amino acids), which are not present in the virus particle, are expressed and play a role in countering the host innate immune response, involved in modulating the host response to infection by inhibiting the induction of the α/β interferon (IFN) in response to viral infection (17-20). RSV appeared to target STAT2 for proteasome-mediated degradation. Depletion of STAT2 would interfere with signaling by IFN- α, β, gama and delta (21).

1.3. Antigenic and genetic subtypes

1.2.1. RSV subtype A and B

Two major antigenic subtypes (subgroups) of the virus, A and B, have been identified (22,23). RSV subtypes are distinguished largely by differences in the viral attachment G protein or the nuclear N protein. G protein...
shows a significant degree of structural and antigenic heterology between subtypes (24). F protein is relatively stable antigenically (5). Antigenic differences among individual strains, especially in the G protein, of the same virus subtype are classed accordingly by monoclonal antibody (Mab) reactivity into antigenic subtypes (23,25-30). During epidemics, either subtype A or B may predominate, or both subtypes may circulate concurrently (31-36). Our previous study monitored the outbreak of RSV infection in Southeast Texas, USA, during the winter season 1991/92, which recovered strains of subtype A and B. The subtype B strains showed 3 patterns of restriction of Mabs against G protein (28). The diversity of its simultaneously circulated RSV strains has made this outbreak unusual. The reason for this is the presence of several antigenic sites of the RSV G protein (37). The existence of distinct lineages within the subtypes has been demonstrated not only on antigenic, but nucleotide level too (38,39). Restriction fragment analysis has identified even greater diversity within subtypes (28,38,40). Genetic differences among RSV strains are determined according to nucleotide sequence and restriction maps of individual gene polymerase chain reaction products (41,42). We investigated the antigenic and genomic characteristics of RSV strains taken from hospitalized children with lower respiratory tract infections in Vienna, Austria, and Zagreb, Croatia, between 1988 and 1994 (43,44). On the criterion of difference in reaction to Mabs, three respective variants of A (A1,A2) and B (B1,B2,B3) RSV subtypes were found circulating in this Central European area in said period. Variant A1 absolutely dominated (Figure 2).

Analysis of the genetic variability of the same RSV strains allowed to discriminate them into five A genotypes (SHL1-5) and one B genotype (NP1) (43,44). The prevalence of different genotypes of RSV in epidemics in Central Europe has shown a pattern similar to those found in the rest of Europe and the world (33,41,43-45). Sequence analysis of the strains from both viral subtypes showed that they share 81% nucleotide identity (5). However, hypervariable regions in G protein show that they are more divergent on the amino acid level than on the nucleotide level (46). This suggests that there is a selective pressure for amino acid substitutions, a pressure that might come from host immunity. It is speculated that these regions in G protein are relatively tolerant of amino acid change because of their unfolded structure. In contrast, although F protein would be subjected to the same selective pressure, it is likely less tolerant of amino acid substitutions due to its folded structure and functional requirements (5). Evidence was provided in favor of progressive viral amino acid changes at an average rate of 0.25% per year (45). Recent study of viruses isolated in Croatia (47) showed that they are closely related to viruses from distinct places (e.g., HR4135-07, HR6010-07, HR2808-07 to NG-009-02 from Japan; HR2671-07 to LLC62-111 and Ab5076Pt01 from Singapore and South Africa respectively; HR263-07 to sal/173/99 from Brazil; all Croatian NA1 strains to NG016-04 and Cam2006-0102 form Japan and Cambodia, respectively; all Croatian BA strains to BA354-04, BA100-04, NG102-05, NG040-07, NG050-09, NG015-09 from Spain and Japan).

1.2.2. Biological characteristics of subtypes

Antigenic variation among RSV isolates may contribute to its ability to cause disease in hosts despite the presence of specific antibodies. Still unproved is the association between the virus-caused clinical picture severity and virus subtype. While the study from Rochester (48) reported that greater severity of RSV diseases in infants was associated with subtype A virus, other studies noted no difference in the severity of illnesses caused by the two subtypes (28,49). In our study from period 2006 to 2007 subtype B caused severe lower respiratory tract infections.

Figure 2. Respiratory syncytial virus subtypes A and B circulated in Zagreb, Croatia and Vienna, Austria from 1988-1994
LRTIs (bronchiolitis and pneumonia) in 58.9% of RSV-patients with infections caused by this subtype (31). Subtype A caused bronchiolitis or pneumonia in 49/94 cases (52.1%, \( p=0.25 \)) (31). Subjects with subtype A or subtype B infection did not differ significantly by age (31) (Figure 3).

1.2.3. Subtype diagnosis

RSV can be diagnosed from patients’ samples by direct or indirect virology methods. Direct methods are performed on respiratory tract samples (e.g. nasopharyngeal secretion-NPS) (50). Virus grows in cell culture (e.g. HEp-2, HeLa, MRC-5) where the cytopathogenic effect of large multinucleated syncytia is formed and, then RSV is identified by immunofluorescent or neutralization test (28,51). Rapid viral detection from NPS can be done by immunofluorescent or molecular method (31). The second one can further subtype isolated or detected RSV strains. Using the molecular method we succeeded in demonstrating that in the Zagreb area, during the period from 2006 to 2008, two different genotypes of subtype A (NA1 and GA5) and three different genotypes of subtype B (BA7, BA9 and BA10) circulated (47). Indirect diagnosis can be done by detecting specific antibodies by neutralization and complement fixation tests. F and G proteins are the only viral proteins that induce neutralizing antibodies (52). Classes (IgM or IgA and IgG) of specific antibodies can be detected from patients’ sera in e.g. enzyme immunoassay (EIA). In EIA-specific antibodies, the response of infants’ sera to purified viral proteins showed that F proteins are 50% antigenically related, as are 1-7% of G proteins (53). The attachment G protein of RSV is associated with disease potentiation and respiratory symptoms through its central conserved gene domain of G gene which inhibits the host innate immune response to RSV and the secretion of inflammatory cytokines by human monocytes (54). In seropositive persons, most viral proteins stimulate RSV-specific memory CD8+ cytotoxic T lymphocytes (55).

1.2.4. RSV proteomic analysis

Today, in the post-genome era, proteomic analysis can provide insights into the complexity of virus-host interactions. The proteome is the entire set of proteins expressed by a genome, cell, tissue or organism. The term, combination of the words protein and genome, was coined by Marc Wilkins in 1994. Proteomic approach has been utilized to investigate proteome changes in cells infected in vitro with different viruses. Two-dimensional gel electrophoresis (2-DE) was used to compare the potential effect of several different enveloped RNA viruses on the host cell proteome (e.g. to study the interaction between RSV and the host cell nuclear proteome) (56,57). Understanding the interaction for RSV and for other viruses with the host cell proteome, will aid in the design of effective antivirals and the development of possible vaccine strategies (58).

References:


