Respiratory syncytial virus

Part I: From genome to proteome analysis

Author: Gordana Mlinaric-Galinovic, MD, PhD

Department of Microbiology University Medical School of Zagreb and Department of Virology, Croatian National Institute of Public Health and, Rockefellerova 12, 10000 Zagreb, Croatia

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).

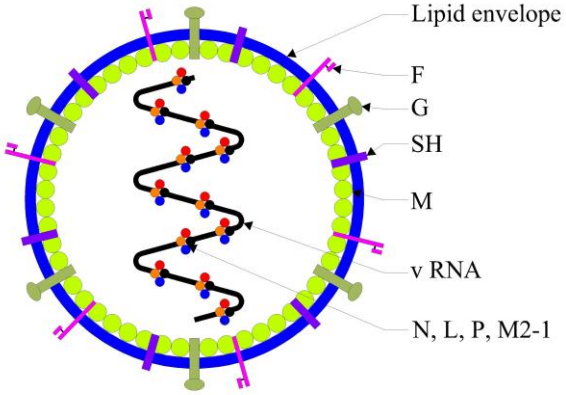

Figure1. Diagram of RSV particle

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 (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).

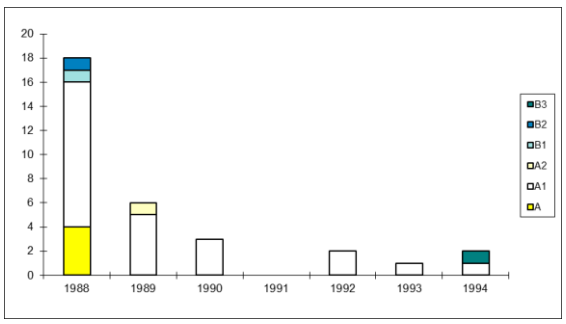

Figure 2. Respiratory syncytial virus subtypes A and B circulated in Zagreb, Croatia and Vienna, Austria from 1988-1994

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

-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).

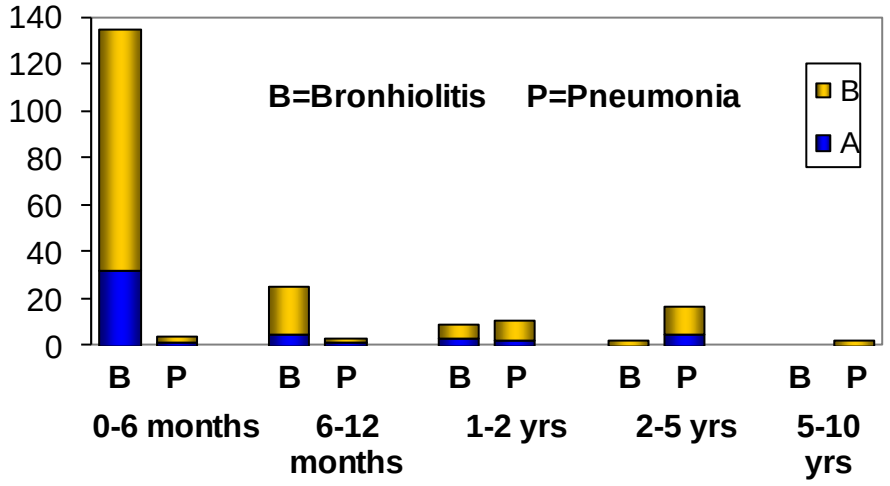

Figure 3. Bronchiolitis and pneumonia (No.) caused by respiratory syncytial virus in Croatia in 2006 and 2007 by viral subtype and age

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:

1. Chanock RM, Finberg L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. Epidemiological aspects of infection in infants and young children. Am J Hyg 1957; 66:291-300.

2. Chanock RM, Roizman B, Myers R. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent. I. Isolation, properties and characterization. Am J Hyg 1957;66:281-90.
3. Gardner PS, Stanfield JP, Wright AE, Court SDM, Green CA: Viruses, bacteria, and respiratory disease in children. British Medical Journal 1960, i: 1077-1081
4. ICTV virus taxonomy version 2009 http://ictvonline.org/virusTaxonomy.asp?version=2009, http://www.ncbi.nlm.nih.gov/ICTVdb/index.htm
5. Collins PL, Crowe JEJr. Respiratory syncytial virus and metapneumovirus. In: Knipe DM, Howley PM, eds. Fields Virology. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2007:1601-1646.
6. Collins LP, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. Journal of Virology. . 2008, 82: 2040–2055.
7. Villanueva N, Hardy RW, Asenjo A, Yu Q, Wertz GW. The bulk of the phosphorylation of human respiratory syncytial virus phosphoprotein is not essential but modulates viral RNA transcription and replication. J Gen Virol 2000, 81:129–133.
8. Hardy RW, Wertz GW. The product of the respiratory syncytial virus M2 gene ORF1 enhances read through of intergenic junctions during viral transcription. J Virol 1998, 72:520–526.
9. Fearns R, Collins PL. Role of the M2-1 transcription antitermination protein of respiratory syncytial virus in sequential transcription. J Virol .1999, 73:5852–5864.
10. Bermingham A, Collins PL. The M2-2 protein of human respiratory syncytial virus is a regulatory factor involved in the balance between RNA replication and transcription. Proc Natl Acad Sci USA 1999, 96:11259–11264.
11. Levine S, Klaiber-Franco R, Paradiso PR. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. J Gen Virol 1987, 68:2521–2524.
12. Martín D, Calder LJ, García-Barreno B, Skehel JJ, Melero JA. Sequence elements of the fusion peptide of human respiratory syncytial virus fusion protein required for activity. J Gen Virol 2006, 87:1649–1658.
13. Carter SD, Dent KC, Atkins E, Foster TL, Verow M, Gorny P, Harris M, Hiscox JA, Ranson NA, Griffin S, Barr JN. Direct visualization of the small hydrophobic protein of human respiratory syncytial virus reveals the structural basis for membrane permeability. FEBS Lett 2010, 584:2786–279.
14. Collins PL, Mottet G. Post-translational processing and oligomerization of the fusion glycoprotein of human respiratory syncytial virus. J Gen Virol 1991; 72:3095-3101.
15. Ghildyal R, Ho A, Jans DA. Central role of the respiratory syncytial virus matrix protein in infection. FEMS Microbiol Rev 2006, 30: 692–700.
16. Tang MN, Collins LP. Identification of the respiratory syncytial virus proteins required for formation and passage of helper-dependent infectious particles. J Virol 1998; 72:5707-5716.
17. Bossert B, Conzelmann KK. Respiratory syncytial virus (RSV) nonstructural (NS) proteins as host range determinants: a chimeric bovine RSV with NS genes from human RSV is attenuated in interferon-competent bovine cells. J Virol 2002, 76: 4287–4293.
18. Bossert B, Marozin S, Conzelmann KK. Nonstructural proteins NS1 and NS2 of bovine respiratory syncytial virus block activation of interferon regulatory factor 3. J Virol 2003, 77:8661–8668.
19. Schlender J, Bossert B, Buchholz U, Conzelmann KK. Bovine respiratory syncytial virus nonstructural proteins NS1 and NS2 cooperatively antagonize alpha/beta interferon-induced antiviral response. J Virol 2000, 74:8234–8240.
20. Spann KM, Tran KC, Collins PL. Effects of nonstructural proteins NS1 and NS2 f human respiratory syncytial virus on interferon regulatory factor 3, NF-kappaB, and proinflammatory cytokines. J Virol 2005; 79:5353-5362.
21. Ramaswamy M, Shi L, Monic MM, Hunninghake GW, Look DC. Specific inhibition of type 1interferon signal transduction by respiratory syncytial virus. Am J Respir Cell Mol Biol 2004;30:893-900.
22. Mufson M, Belshe R, Orvell C, Norrby E. Respiratory syncytial virus epidemics: variable dominance of subgroups A and B strains among children, 1981-1986. Journal of Infectious Diseases 1988, 157:143-148.
23. Anderson LJ, Hierholzer JC, Tsou C, Hendry RM, Fernie BF, Stone Y, McIntosh K. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. Journal of Infectious Diseases 1985. 151:626-633.
24. 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.
25. Mufson MA, Orvell C, Rafnar B, Norrby E: Two distinct subtypes of human respiratory syncytial virus. Journal of General Virology 1985, 66: 2111-2124.
26. Anderson LJ, Hendry RM, Pierik LT, Tsou C, McIntosh K. Multicenter study of strains of respiratory syncytial virus. J Infect Dis 1991;163:687-92.
27. Hall CB, Walsh EE, Schnabel KC, Long CE, McConnochie KM, Hildreth SW, Anderson LJ. Occurrence of groups A and B of respiratory syncytial virus over 15 years: associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. J Infect Dis 1990;162:1283-90.
28 Mlinaric-Galinovic G, Chonmaitree T, Cane PA, Pringle CR, Ogra PL: Antigenic diversity of respiratory syncytial virus subgroup B strains circulating during a community outbreak of infection. Journal of Medical Virology 1994, 42: 380-384.
29. Johnson PR Jr, Olmstead RA, Prince GA, Murphy BR, Alling DW, Walt EE, Collins PL: Antigenic relatedness between glycoproteins of human respiratory syncytial virus subgroups A and B: evaluation of the contributions of F and G glycoproteins to immunity. Journal of Virology 1987, 61: 3163-3166.
30. Akerlind B, Norrby E, Orvell C, Mufson MA: Respiratory syncytial virus: heterogeneity of subgroup B strains. Journal of General Virology 1988, 69: 2145-2154.
31. Mlinaric-Galinovic G, Vojnovic G, Bogovic-Cepin J, Bace A, Bozikov J, Welliver RC, Wahn U, Cebalo L . Does the viral subtype influence the biennial cycle of respiratory syncytial virus? Virol J 2009, 6 (1):133-139.
32. Zlateva, K. T., Vijgen L, Dekeersmaeker N, Naranjo C, Van Ranst M**.** Subgroup prevalence and genotype circulation patterns of human respiratory syncytial virus in Belgium during ten successive epidemic seasons. J. Clin. Microbiol 2007**;** 45:3022-3030.

33. Peret TCT, Hall CB, Schnabel KC, Golub JA, Anderson LJ. Circulation pattern of genetically distinct group A and B strains of human respiratory syncytial virus in a community. *Journal of General Virology* 1998, 79: 2221-2229.
34. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, Auinger P, Griffin MR, Poehling KA, Erdman D, Grijalva CG, Zhu Y, Szilagyi P. The burden of respiratory syncytial virus infection in young children. The New England Journal of Medicine 2009, 360:588-598.
35. Shobugawa Y, Saito R, Sano Y, Zaraket H, Suzuki Y, Kumaki A, Dapat I, Oguma T, Yamaguchi M, Suzuki H. Emerging genotypes of human respiratory syncytial virus subgroup A among patients in Japan. J. Clin Microbiol 2009; 47:2475-2482.
36. Zhang ZY, Du LN, Chen X, Zhao Y, Liu EM, Yang XQ, Zhao XD .Genetic variability of respiratory syncytial viruses (RSV) prevalent in Southwestern China from 2006 to 2009: emergence of subgroup B and A RSV as dominant strains. J Clin Microbiol 2010; 48(4):1201-7.
37. Walsh EE, Hall CB, Schlesinger JJ, Brandriss MW, Hildreth S, Paradiso P. Comparison of antigenic sites of subtype-specific respiratory syncytial virus attachment proteins. J Gen Virol 1989,70:2953-2961.
38. Sullender WM, Sun L, Anderson LJ: Analysis of respiratory syncytial virus genetic variability with amplified cDNAs. Journal of Clinical Microbiology 1993, 31: 1224-1231.
39. Cane PA, Pringle CR. Molecular epidemiology of respiratory syncytial virus: a review of the use of reverse transcription-polymerase chain reaction in the analysis of genetic variability, Electrophoresis 1995, 16:329-333.
40. Sullender WM, Anderson LJ, Anderson K, Wertz GW: Differentiation of respiratory syncytial virus subgroups with cDNA probes in a nucleic acid hybridization assay. Journal of Clinical Microbiology 1990, 28: 1683 -1687.
41. Cane PA, Matthews DA, Pringle CR. Analysis of relatedness of subgroup A respiratory syncytial viruses isolated worldwide. Virus Res 1992, 25:15-22.
42. Cane PA, Pringle CR. Respiratory syncytial virus heterogeneity during an epidemic: analysis by limited nucleotide sequencing (SH gene) and restriction mapping (N gene). J Gen Virol 1991, 72:349-357.
43. Lukic-Grlic A, Cane PA, Bace A, Pringle CR, Mlinaric-Galinovic G, Popow-Kraupp T. Antigenic and genomic diversity of central European respiratory syncytial virus strains. Archives of Virology 1998, 143:1441-1447.
44. Lukic-Grlic A, Mlinaric-Galinovic G: Podtipovi respiratornog sincicijskog virusa. Lijec Vjesn 121: 144-147, 1999.
45. Cane PA, Pringle CR. Evolution of subgroup A respiratory syncytial virus: evidence for progressive accumulation of amino acids changes in the attachment protein. J Virol 1995; 69:2918-2925.
46. Johnson PP, Spriggs MK, Olmsted RA, Collins PL. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc Natl Acad Sci U S A 1987; 84:5625-5629.
47. Forcic D, Ivancic-Jelecki J, Mlinaric-Galinovic G, Vojnovic G, Babic-Erceg A, Tabain I: A study of the genetic variability of human respiratory syncytial virus in Croatia, 2006-2008. J Med Virol 2012, 84(12):1985-92.
48. McConnochie KM, Hall CB, Walsh EE, Roghmann KJ: Variation in severity of respiratory syncytial virus infections with subtype. Journal of Pediatrics 1990, 117:52-62.
49. Hendry RM, Pierik LT, McIntosh K: Prevalence of respiratory syncytial virus subgroups over six consecutive outbreaks: 1981-1987. Journal of Infectious Diseases 1989, 160:185-190.
50. Mlinaric-Galinovic G, Grljusic V: Nazofaringealni sekret bolesnika kao klinicki materijal kod virusnih respiratornih infekcija. Lijec Vjesn 1989, 111: 213-214.
51. Welliver RC, Ogra PL. Respiratory syncytial virus. In: Gorbach SL, Bartlett JG, Blacklow NR (eds). Infectious Diseases, 3rd ed, Lippincott Williams & Wilkins, New York, 2003; 2031-8.
52. Connors M, Collins Pl, Firestone CY, Murphy BR. Respiratory syncytial virus (RSV) F, G, M2 (22K), and N proteins each induce resistance to RSV challenge, but resistance induced by M2 and N proteins is relatively short-lived. J Virol 1991; 65:1634-1637.
53. Hendry RM, Burns JC, Walsh EE, Graham BS, Wright PF, Hemming VG, Rodriguez WJ, Kim HW, Prince GA, McIntosh K, Chanock RM, Murphy BR. Strain-specific serum antibody responses in infants undergoing primary infection with respiratory syncytial virus. J Infect Dis 1988; 157:640-647.
54. Polack FP, Irusta PM, Hoffman SJ, Schiatti MP, Melendi GA, Delgado MF, Laham FR, Thumar B, Hendry RM, Melero JA, Karron RA, Collins PL, Kleeberger SR. The cysteine-rich region of respiratory syncytial virus attachment protein inhibits innate immunity elicited by the virus and endotoxin. Proc Natl Acad Sci U S A 2005; 102: 8996-9001.
55. Cherrie AH, Anderson K, Wertz GW, Openshaw PJ. Human cytotoxic T cells stimulated by antigen on dendritic cells recognize the N, SH, F, M, 22K, and 1b proteins of respiratory syncytial virus J Virol 1992; 66:2102-2110.
56. Brasier AR, Spratt H, Wu Z, Boldogh I, Zhang Y, Garofalo RP, Casola A, Pashmi J, Haag A, Luxon B, Kurosky A. Nuclear heat shock response and novel nuclear domain 10 reorganization in respiratory syncytial virus-infected A549 cells identified by high-resolution two-dimensional gel electrophoresis, J Virol 2004, 78: 11461–11476.
57. Munday DC, Emmott E, Surtees R, Lardeau CH, Wu W, Duprex WP, Dove BK, Barr JN, Hiscox JA: Quantitative proteomic analysis of A549 cells infected with human respiratory syncytial virus. Molecular & cellular proteomics: MCP 2010, 9:2438-2459.
58. Collins PL, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. J Virol 2008, 82:2040–2055.