Molecular epidemiology of respiratory viruses in virus-induced asthma

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INTRODUCTION

Acute respiratory illness (ARI) due to various viruses is not only the most common cause of upper respiratory infection in humans but is also a major cause of morbidity and mortality, leading to diseases such as bronchiolitis and pneumonia. Previous studies have shown that respiratory syncytial virus (RSV), human rhinovirus (HRV), human metapneumovirus (HMPV), human parainfluenza virus (HPIV), and human enterovirus infections may be associated with virus-induced asthma. For example, it has been suggested that HRV infection is detected in the acute exacerbation of asthma and infection is prolonged. Thus it is believed that the main etiological cause of asthma is ARI viruses. Furthermore, the number of asthma patients in most industrial countries has greatly increased, resulting in a morbidity rate of around 10–15% of the population. However, the relationships between viral infections, host immune response, and host factors in the pathophysiology of asthma remain unclear. To gain a better understanding of the epidemiology of virus-induced asthma, it is important to assess both the characteristics of the viruses and the host defense mechanisms. Molecular epidemiology enables us to understand the pathogenesis of microorganisms by identifying specific pathways, molecules, and genes that influence the risk of developing a disease. However, the epidemiology of various respiratory viruses associated with virus-induced asthma is not fully understood. Therefore, in this article, we review molecular epidemiological studies of RSV, HRV, HPIV, and HMPV infection associated with virus-induced asthma.

Keywords: molecular epidemiology, virus-induced asthma, respiratory syncytial virus, human rhinovirus, human metapneumovirus, respiratory viruses

Acute respiratory illness (ARI) is a major cause of morbidity and mortality worldwide (Williams et al., 2002; Sloots et al., 2008). ARI imposes a huge burden on health, particularly in children. For community-based care, ARI has been estimated at a cost of over US$100 per case (Ehlken et al., 2005). The disease burden for ARI comes in patients with established asthma and are associated with nearly 80% of asthma exacerbation episodes (Nicholson et al., 1993; Johnston et al., 1995; Wark et al., 2002; Heymann et al., 2004; Grissell et al., 2005). Accumulating evidence indicates that the etiology of most cases of asthma, namely virus-induced asthma, is linked to such respiratory virus infections. In addition, RSV and HRV are the most frequently detected pathogens and may play an important role in viral induction and exacerbation of asthma.

Although severe lower respiratory tract infections have been observed, ARI is most often associated with mild upper respiratory infection (URI). Most ARI cases in early childhood are confirmed as URI, leading to symptoms of the common cold with coryza and cough. In contrast, around one-third of infants with ARI develop lower respiratory tract symptoms such as tachypnea, wheezing, severe cough, breathlessness, and respiratory distress (Treganion and Schwarze, 2010). In general, viruses are the most common causative agents of ARI. More than 200 different types of viruses are known to cause ARI, with respiratory syncytial virus (RSV), human rhinovirus (HRV), human metapneumovirus (HMPV), and human parainfluenza virus (HPIV) most commonly identified in ARI patients. Indeed, together with these respiratory viruses, human enterovirus (HEV), influenza virus (InfV), human coronavirus (HCoV), adenovirus (Adv), and human bocavirus (HBoV) account for around 70% of ARIs detected (Kusel et al., 2006). Respiratory viral infections can have severe adverse outcomes in patients with established asthma and are associated with nearly 80% of asthma exacerbation episodes (Nicholson et al., 1993; Johnston et al., 1995; Wark et al., 2002; Heymann et al., 2004; Grissell et al., 2005). Accumulating evidence indicates that the etiology of most cases of asthma, namely virus-induced asthma, is linked to such respiratory virus infections. In addition, RSV and HRV are the most frequently detected pathogens and may play an important role in viral induction and exacerbation of asthma.

VIRAL INFECTION AND ASThma

In infancy, illnesses such as bronchiolitis share many clinical features with acute asthma, including wheezing, rapid breath-
compromise. Respiratory viruses are detected in the majority of asthma exacerbations in both children (80–85%) and adults (75–80%; Johnston et al., 1995; Grissell et al., 2005). In addition, wheezing illnesses are also closely associated with respiratory viral infections in all age groups (Gern, 2010). Fujitsuka et al. (2011) hypothesized that such respiratory virus infections are deeply associated with virus-induced asthma (Kusel et al., 2007; Pierangeli et al., 2007; Kuehni et al., 2009). Thus, it is entirely plausible that viral infections induction and/or exacerbation asthma in children.

MOLECULAR EPIDEMIOLOGY OF RSV

Respiratory syncytial virus of genus Pneumovirus and family Paramyxoviridae causes ARI in children (Varlas et al., 1999; Peter and James, 2006). RSV infection may cause major problems in infants less than 1 year of age and can lead to life-threatening ARIs such as bronchiolitis and bronchopneumonia (Shay et al., 1999; Leung et al., 2005; Yorita et al., 2007). Epidemiological studies suggest that around 70% of infants have experienced an RSV infection by the age of 1 year, and 100% by the age of 2 years; host response to the virus varies greatly, but includes upper respiratory tract infections, typical bronchiolitis, and RSV-induced wheezy bronchitis (Cane, 2001; Kuehni et al., 2009). Long-term prospective case-control and cohort studies have also linked RSV bronchiolitis to the development of wheezing and asthma later in childhood (Sigurs et al., 1995, 2005, 2010; Henderson et al., 2005). Thus, RSV infections may be associated with the initiation and/or exacerbation of asthma.

The RSV genome encodes 11 proteins (Peter and James, 2006). Among these, the attachment glycoprotein (G) is a major structural protein that may be associated with both infectivity and antigenicity (Johnson et al., 1987; Rueda et al., 1991). Molecular epidemiological studies have shown that RSV can be classified into two phylogenetic subgroups, RSV-A and RSV-B (Mufson et al., 1985). The strains of subgroup A can be subclassified into eight genotypes (GA1–GA7 and SAA1), as can those of subgroup B (BA, GB1–GB4, and SAB1–3; Parveen et al., 2006). From phylogenetic analysis of the G gene of RSV, Martinello et al. (2002) showed that RSV belonging to GA3 genotype may be associated with greater severity of illness in, for example, bronchiolitis and pneumonia. Although GA3 genotype has been detected in the United Kingdom, Spain, and New Zealand, it is not the most prevalent strain (Cane et al., 1991; Garcia et al., 1997; Matheson et al., 2006). Martinello et al. (2002) therefore suggested that the association between greater severity of illness and GA3 genotype may be solely due to a transient shift in genotype-specific immune status within the community. In addition, correlations between certain strains and/or genotypes of RSV and slight differences in disease severity have been described previously (Hall et al., 1990; Wald et al., 1997). Some genotypes such as subgroup A genotypes GA1, GA2, GA5, GA7, and NA1 and subgroup B genotype BA have been detected throughout the world in recent years (Zlateva et al., 2004; Parveen et al., 2006; Zhang et al., 2007; Nakamura et al., 2009; Rebuffs-Scheir, 2011). Of these, NA1 is a novel genotype known to be genetically close to GA2 genotype, while GA2 genotype and BA genotype are the most common genotypes of RSV subgroups A and B around the world and have persisted for many years (Tit et al., 2013). Furthermore, a new genotype belonging to RSV-A, ON1, has been detected in some countries, including...
MOLECULAR EPIDEMIOLOGY OF HRV

Human rhinovirus is a group of positive-sense sRNA viruses belonging to genus *Enterovirus* in the family *Picornaviridae* (Turner and Couch, 2007). Although HRVs were previously thought to be mainly associated with the common cold causing mild respiratory symptoms, recent reports strongly suggest that HRVs may induce and/or exacerbate asthma (virus-induced asthma; Chung et al., 2007; Turner and Couch, 2007; Busse et al., 2010). One report suggested that HRV wheezing illness within the first three years of life is significantly associated with the development of asthma at age 6 years (Jackson et al., 2008). Further research is needed to clarify the role of HRVs in the development of asthma.

In children with rhinovirus-induced ARI, the severity of illness is not linked to subgroups or serotypes, but is associated with the quantity of RSV in nasopharyngeal aspirate (Sullender, 2000; Campanini et al., 2007). A larger population study is needed to identify the different RSV genotypes circulating in different areas to gain a better understanding of the relationship between disease severity and RSV genotype.

The G protein is a major antigen of RSV and amino acid substitutions may be related to changes in antigenicity. There are some reports of amino acid substitutions, and some positively selected sites in the G-terminal 3rd hypervariable region of the G gene have been estimated (Botosso et al., 2009; Yoshida et al., 2012; Kushibuchi et al., 2013). For example, Yoshida et al. (2012) estimated some positive selection in the region (Asn250Ser, Met262Glu, Arg297Lys, and Arg297Glu substitutions in RSV-A strains were estimated by the REL method, and Asn273Tyr and Leu274Pro substitutions of RSV-A, as well as Leu273Pro substitution of RSV-B, were estimated by the IFEL method). Botosso et al. (2009) found 29 and 23 amino acid sites under positive selection in RSV-A and RSV-B, respectively. In addition, some unique positively selected sites were found in the G gene (Kushibuchi et al., 2013). These amino acid variations at these sites might play a key role in severe respiratory infection, such as bronchiolitis (Goto-Sugai et al., 2010). Furthermore, the rate of molecular evolution of the region might be high. For example, Kushibuchi et al. (2013) estimated the evolutionary rate of RSV-A at 3.63 × 10⁻³ substitutions/site/year, while that of RSV-B was estimated at 4.56 × 10⁻³ substitutions/site/year. Thus, it is suggested that this C-terminal 3rd hypervariable region in the G gene of RSV-A and -B evolved rapidly (Kushibuchi et al., 2013). Based on host immunological conditions, it is suggested that host immunity such as TLR4 polymorphism is linked to symptomatic RSV infection (Delgado et al., 2009). Thus, both the antigenicity of the viruses and host immune conditions may play important roles in the pathophysiology of severe respiratory infections such as bronchiolitis, pneumonia, and virus-induced asthma (Asoomoyi et al., 2007).

The genetic diversity of RSV-A and -B strains was estimated by the REL method, and Asn273Tyr and Leu274Pro substitutions of RSV-A, as well as Leu273Pro substitution of RSV-B, were estimated by the IFEL method. Botosso et al. (2009) found 29 and 23 amino acid sites under positive selection in RSV-A and RSV-B, respectively. In addition, some unique positively selected sites were found in the G gene (Kushibuchi et al., 2013). These amino acid variations at these sites might play a key role in severe respiratory infection, such as bronchiolitis (Goto-Sugai et al., 2010). Furthermore, the rate of molecular evolution of the region might be high. For example, Kushibuchi et al. (2013) estimated the evolutionary rate of RSV-A at 3.63 × 10⁻³ substitutions/site/year, while that of RSV-B was estimated at 4.56 × 10⁻³ substitutions/site/year. Thus, it is suggested that this C-terminal 3rd hypervariable region in the G gene of RSV-A and -B evolved rapidly (Kushibuchi et al., 2013). Based on host immunological conditions, it is suggested that host immunity such as TLR4 polymorphism is linked to symptomatic RSV infection (Delgado et al., 2009). Thus, both the antigenicity of the viruses and host immune conditions may play important roles in the pathophysiology of severe respiratory infections such as bronchiolitis, pneumonia, and virus-induced asthma (Asoomoyi et al., 2007).

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MOLECULAR EPIDEMIOLOGY OF HMPV

Human metapneumovirus is a recently identified RNA virus belonging to the Paramyxoviridae family, of genus Metapneumovirus (Collins and Crowe, 2007). HMPV is a major pathogen that causes ARI in all ages (Collins and Crowe, 2007). The first HMPV infection appears to take place within the first six months of life, after which infections may occur repeatedly and frequently (Schildgen et al., 2011). The nosocomial impact of HMPV is estimated to be as high as that for RSV. In an HMPV outbreak in Japan, 34.8% of elderly patients that shared the same day care room in a hospital were infected with HMPV (Honda et al., 2006). Higher morbidity is observed in young children, the elderly, and immunocompromised adults (Beuron et al., 2002; Faley et al., 2003; van den Hoogen et al., 2003; Sumino et al., 2005, Williams et al., 2005; O’Gorman et al., 2006). HMPV is classified into two genotypes (A and B) and four subgroups (A1, A2, B1, and B2) by phylogenetic analysis, using the F and G genes (Biacchi et al., 2003; van den Hoogen et al., 2004). Subgroup A2 has been subdivided into two lineages, subgroup A2a and A2b (Huck et al., 2006). It has been suggested that these genotypes circulate in variable proportions in some areas (Gerna et al., 2005; Mackay et al., 2006). Although the molecular epidemiological information on HMPV has gradually accumulated, the detailed epidemiology remains unclear (Mizuta et al., 2010b; Pitoiset et al., 2010; Omura et al., 2011). HMPV infections can occur throughout the year, but seasonality has been described in several studies, with the epidemiological peak occurring several months later than that observed for RSV epidemics (Robinson et al., 2005; Wilkesmann et al., 2006; Madhi et al., 2006). Henrickson and Savatski (1996) analyzed the longitudinal evolution of the HN coding region in 13 strains of HPIV1 isolated in the USA. These results showed that the antigenic and genetic subgroups are very stable. In addition, Mizuta et al. (2013) suggested that four different types of HPIV cause similar clinical manifestations in patients, and the clinical presentation of HPIV infection may differ depending on patient age (Liu et al., 2013). Henrickson and Savatski (1996) analyzed the longitudinal evolution of the HN coding region in 13 strains of HPIV1 isolated in the USA. These results showed that the antigenic and genetic subgroups are very stable. In addition, Mizuta et al. (2013) suggested that the evolution of the HN gene in the present HPIV1 isolates was relatively slow and that the gene is highly conserved. Only a few reports on the molecular epidemiology of HPIV1 are available and it appears that the molecular epidemiology of HPIV is poorly understood. Larger and more detailed studies on the association of HPIV with asthma are needed.

MOLECULAR EPIDEMIOLOGY OF OTHER VIRUSES

HEV68 was recently detected in asthmatic patients (Hasegawa et al., 2013). HEV68 was found to be relatively acid resistant and thus could be distinguished from acid-sensitive HRV87 (Schieble et al., 1967; Kapikian et al., 1971). HRV87 was recently reclassified...
as HEV68 based on phylogenetic analysis and neutralization test, and some laboratories have confirmed its acid sensitivity (Blomqvist et al., 2002; Ishiko et al., 2002; Savolainen et al., 2002). Distinguishing between HRV and HEV based on the acid sensitivity of isolates is therefore not appropriate for HEV68. The number of reports of an association between respiratory disease and HEV68 infection has recently increased. One report of the phylogenetic analysis of HEV68 based on partial VP1 gene sequences indicates wide genetic diversity (Linskasvaino et al., 2012). In addition, Tokars et al. (2012) showed the presence of multiple clades among the circulating strains, and that all strains are spreading rapidly worldwide and contributing to the prevalence rates of respiratory diseases. In addition, asthmatic individuals infected with HEV68 also have the propensity to develop unstable asthma or an acute attack (Hasegawa et al., 2011).

Influenza virus is also a major causative agent of ARI in both children and adults. Furthermore, asthmatic patients were found among children and adults hospitalized with seasonal InfV (Dao et al., 2010; Dawood et al., 2010). Although it is recognized that viral infections such as RSV or HRV may induce and/or exacerbate asthma, the effect of InfV on asthma remains arguable (Johnston et al., 1999). Although one study suggested that A(H1N1)pdm09 viruses impose greater risk factors on children than seasonal InfV (Tian et al., 2012), InfV vaccine was available before the influenza season since InfV causes more severe illness than other respiratory viruses. Therefore, it is suggested that InfV vaccine be recommended for children with asthma (Kloepfer et al., 2012). Although the level of detection of HCoV, HRV, or AdV is relatively low, these infections are also detected in children with acute wheezing (Chung et al., 2007; Jariit et al., 2007). Further studies are needed to clarify the clinical roles of HCoV, HRV, or AdV infections and those of other respiratory viruses. In particular, the prevalence of HCoV, HRV, or AdV infection in healthy control subjects, assessment of disease severity by other clinical variables, and the immunological effects should be investigated.

MOLECULAR EPIDEMIOLOGY OF CO-INFECTION

Infants with severe bronchiolitis have an increased risk of developing recurrent wheezing later in life (Chung et al., 2007). HRV may be detected concurrently with other viruses such as RSV, HCoV, HEV68 (Abrole et al., 2008; Fujitsu et al., 2011). Considering their ubiquity, it is interesting that the number of respiratory viruses detected concurrently with HRV strains is relatively low (Lamber et al., 2007; Mackay, 2007), supporting the concept that HRVs have a direct role in the clinical outcome of infection (Miller et al., 2007). In fact, HRV strains are co-detected with other pathogens in reproducible, but clinically undefined, patterns (Beaujean et al., 2008). The HRV partnership with host immunity may be a mutalistic one, inadvertently imparting an advantage to the host by protecting against more cytotoxic respiratory viral pathogens while the host provides a vessel for HRV replication and transmission.

Respiratory viruses other than RSV and multiple viral infections may contribute to the severity of bronchiolitis and asthma. Indeed, it was reported that dual infections of HMPV and RSV or HRV and RSV confer a 5- to 10-fold increase of severe illness compared to children admitted to pediatric intensive care units (Papadopoulos et al., 2002; Semple et al., 2005). In contrast, other studies reported that co-infection with two respiratory viruses was not significantly associated with disease severity (van Woensel et al., 2006; Wolf et al., 2006). Thus, there is no consensus on the effects of co-infection on disease severity. The effect of dual infection may depend upon which viruses co-infect together. For example, although there was no increase in severity when HRV and/or AdV were detected during RSV infection, co-infection with both HMPV and RSV increased the rate of intensive care unit admissions (Abrole et al., 2005; Semple et al., 2005). Thus, although dual infections and reinfections have been well documented in children, chronic infection with the development of quasispecies cannot be ruled out without obtaining more complete data using high performance detection methods (Hall and McCarthy, 1996).

CONCLUSION

Respiratory viral infections are a major cause of virus-induced asthma in early life. Although antiviral therapy is not yet available for patients infected with respiratory viruses, the detection and identification of these viruses could help to explain serious respiratory illness, provide guidance for medical care, and prevent unnecessary treatment with antibiotics. Based on the results of many related studies, we propose a two-step hypothesis of asthma development in children. The first step is mainly due to RSV infection: when RSV infects bronchial cells, the bronchial cells produce various cytokines and chemokines. These responses cause hyperreactiveness in the bronchial cells. In other words, RSV infection might create a preparatory step as the first step in the development of asthma. HRV infection might then bring about the second step in the development of asthma. An infant with a history of wheezing caused by RSV infection may develop the heavy wheezing of asthma due to HRV infection followed by RSV infection. To understand the cause of asthma, we need to examine the larger complex picture of genetic susceptibility, immune components, environmental exposures, and the interactions between these elements.

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REFERENCES

Abrole, J. H., Abrole, S. W., Pracher, E., Hunter, H. P., Kudari, M., and Popow-Kraapp, T. (2005). Single versus dual respiratory virus infections in hospitalized infant: impact on clinical course of disease and infection-gamma response. Pediatr Infect. Dis. J. 24, 605–610. doi:10.1097/00006454-200507000-00008. Abrole, J. H., Abrole, S. W., Bullberger-Fritze, M., Sandhofer, M. J., and Popow-Kraapp, T. (2008). Human metapneumovirus subgroup changes and vasoactivity during epidemics. Pediatr Infect. Dis. J. 29, 1016–1018.

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Arakawa, M., Okamoto-Nakagawa, R., Toda, S., Tsukagoshi, H., Kominami, M., Ito, A., et al. (2012). Molecular epidemiological study of rhinovirus species A, B and C from patients with acute respiratory illnesses. J. Med. Microbiol. 61, 410–419. doi: 10.1099/jmm.0.075006-0
Arnot, A., Yong, S., Kaul, M.A., Naughtin, M., Buxton, J., Rohl, S., et al. (2011). Genetic variability of human metapneumovirus strains circulating amongst an age-matched population in Cambodia between 2007 and 2009. Infect. Genet. Evol. 11, 43–52. doi: 10.1016/j.meegid.2010.11.014
Aronesty, A. A., Rallabhandi, P., Pullin, T. L., Lorenz, E., Steven, M. B., Redmond, M. S., et al. (2007). Association of TLK polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children. J. Infect. 57, 371–377.
Awomoyi, A. A., Rallabhandi, P., Arnott, A., Vong, S., Sek, M., Boivin, G., Abed, Y., Pelletier, G., Blomqvist, S., Savolainen, C., Råman, B. R., Collins, P. L., et al. (2003). Positive selection and diversity among respiratory syncytial virus A subgroup C strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. J. Gen. Virol. 84, 265–274. doi: 10.1099/0022-1317-83-1-265
Awomoyi, A. A., Rallabhandi, P., Arnott, A., Vong, S., Sek, M., Boivin, G., Abed, Y., Pelletier, G., Blomqvist, S., Savolainen, C., Råman, B. R., Collins, P. L., et al. (2003). Positive selection and diversity among respiratory syncytial virus A subgroup C strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. J. Gen. Virol. 84, 265–274. doi: 10.1099/0022-1317-83-1-265
Aronesty, A. A., Rallabhandi, P., Pullin, T. L., Lorenz, E., Steven, M. B., Redmond, M. S., et al. (2007). Association of TLK polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children. J. Infect. 57, 371–377.
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Hall, C. B., Wobbe, E. E., Schmale, K. C., Long, C. E., McCombie, K. M., Hildroff, S. W., et al. (1980). Occurrence of groups A and B of respiratory syncytial virus over 15 years: associated epidemiology and clinical characteristics in hospitalized and ambulatory children. J. Infect. Dis. 142, 1285–1290. doi: 10.1093/infdis/142.3.1285

Hasegawa, S., Hirasaka, K., Nakazato, K., Shiota, T., and Shiraki, K. (2011). Enterovirus 68 infection in children with asthma attacks virus-induced asthma in Japanese children. Allergy 66, 1618–1623. doi: 10.1111/j.1398-9995.2011.02725.x

Henninger, U., Kruco, A. T., Brohoffer, H., and Schad, U. R. (2009). Human metapneumovirus infections –bianetral evidence and clinical findings in children in the region of Basel, Switzerland. Z. J. Pediatr. 158, 1455–1460. doi: 10.1007/s00431-009-0486-0

Henrickson, K. J. (2003). Parainfluenza virus type 1 genotypes detected during the 1991 rhinorrhea epidemic. Clin. Microbiol Rev. 16, 829–880. doi: 10.1128/CMR.16.4.829-880.2003

Henrickson, K. J., and Saravolatz, L. L. (1996). Two distinct human parainfluenza virus type 1 genotypes associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatr. Infect. Dis. 15, 1738–1744. doi: 10.1097/00006454-199608000-00022

Jackson, D. J., Gangnon, R. E., Evans, M. D., Robberg, K. A., Anderson, E. L., Dppas, T. E., et al. (2000). The human rhinovirus 14, 1275–1280. doi: 10.1016/S1473-3099(00)00272-1

Johnson, P. R., Spriggs, M. K., Jennings, L. C., Anderson, T. P., Beynon, L. L., and Collins, P. R. (1987). The G glycoprotein of G3 parainfluenza virus. Proc. Natl. Acad. Sci. U.S.A. 84, 5625–5629. doi: 10.1073/pnas.84.16.5625

Johnson, P. F., Patterson, J. M., Sandersen, G., Smith, S., Lampie, P., Joseph, D. L. (1995). Community-wide study of respiratory infections in exacerbations of asthma in 9–11 year old children. BMJ 310, 1225–1229. doi: 10.1136/bmj.310.6997.1225

Jovan, T., Mirsola, J., Wair, M., Lottenberg, M., and Ruuskanen, O. (2004). Clinical evidence for antibiotic therapy for community-acquired pneumonia. Eur. J. Pediatr. 163, 140–144. doi: 10.1007/s00431-003-1387-2

Kaida, A., Iritani, N., Kudo, H., Shimizu, M., Ichikura, U., and Murakami, T. (2006). Seasonal distribution and phylogenetic analysis of human metapneumovirus among children in Osaka City, Japan. J. Clin. Virology 35, 394–399. doi: 10.1016/j.jcv.2005.12.009

Kapikian, A. Z., Conant, R. M., Lumpe, H., and Platt, M. H. (1971). A collaborative report: rhinovirus – examination of the strain system. Pediatrics 48, 534–541. doi: 10.1542/peds.48.3.534

Karron, R. A., and Belshe, B. (1997). “Parainfluenza virus,” in Fields Virology, Vol. 5, 1st Edn, eds T. H. V. Fields, D. M. Knipe and P. M. Howley (Philadelphia: Lippincott Williams & Wilkins), 1497–1526.

Kato, M., Tsukagoshi, H., Yoshizumi, M., Satoh, M., Konara, K., Yamada, Y., et al. (2011). Different cytokine profile and eosinophil activation are involved in rhinovirus- and RSV-induced acute exacerbation of childhood polyvalent. Pediatr. Allergy Immunol. 22, 487–494. doi: 10.1111/j.1399-3038.2010.01305.x

Khadadah, M., Essa, S., Higazi, Z., Babacheri, N., and Ali-Nakhl, W. (2010). Respiratory syncytial virus and human rhinovirus are the major causes of severe lower respiratory tract infections in Kuwait. J. Med. Virol. 82, 1462–1471. doi: 10.1002/jmv.21825

Khosla, C. S., Sam, I. C., Hoe, P. S., and Chan, Y. F. (2013). Displacement of predominant respiratory virus genotypes in Malaysia between 1989 and 2011. J. Infect. Dev. Ctries. 7, 316–320. doi: 10.3855/jidc.2012.12.017

Kroksa, N., Kushibuchi, I., Kusaka, M., Tsukagoshi, H., Ito, A., Nonohara, K., et al. (2013). Genetic analysis of the VP4/VP6 coupling region in human rhinovirus strain C in patients with acute respiratory infection in Japan. J. Med. Microbiol. 62, 616–617. doi: 10.1099/jmm.0.049702-0

Kloeper, K. M., Okoscie, J. P., Lee, W. M., Liu, G., Vin, R. B., Robberg, K. A., and Thiemig, V. E. (2012). Increased H1N1 infection rate in children with asthma. Am. J. Respir. Crit. Care Med. 185, 1497–1505. doi: 10.1164/rccm.201109-1397OC

Knott, A. M., Long, C. E., and Hall, C. B. (1994). Parainfluenza viral infections in pediatric outpatient seasonal patterns and clinical characteristics. Pediatr. Infect. Dis. J. 13, 269–273. doi: 10.1097/00006454-199409000-00005

Kusuhara, C. E., Spruill, B. D., and Silverman, M. (2009). Causal link between RSV infection and asthma: no clear answers to an old question. Am. J. Respir. Crit. Care Med. 179, 1079–1088. doi: 10.1164/rccm.200904-0572ED

Kuo, M. M., de Klerk, N. H., Kabade, T., Volmink, V., Ehto, P. G., John- ston, S. L., and Sy, P. D. (2006). Role of respiratory viruses in acute lower and respiratory tract illness in the first year of life: a birth cohort study. Pediatr. Infect. Dis. J. 25, 600–606. doi: 10.1097/01.inf.0000242612.88906.a5

Kuusela, M. M., de Klerk, N. H., Kabade, T., Volmink, V., Ehto, P. G., Johnston, S. L., and Sy, P. D. (2007). Early life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J. Allergy Clin. Immunol. 119, 1105–1111. doi: 10.1016/j.jaci.2006.12.069

Kushibuchi, I., Kusaka, M., Kusaka, T., Tsukagoshi, H., Ito, A., Yoshida, A., et al. (2013). Molecular evolution of attachment glycoprotein (s) gene in human respiratory syncytial virus detected in Japan 2008–2011. J. Infect. Dev. Ctries. 168–173. doi: 10.1016/j.jcid.2013.05.010

Lambert, S. B., Allen, K. M., Dwae, J. D., Birch, C. J., Mackay, I. M., Carlin, J. B., et al. (2007). Community epidemiology of human metapneumovirus, human coronavi- rus NL63, and other respiratory viruses in healthy pre-school-aged children: a population-based study. Pediatr. Pulmonol. 42, e29–e37. doi: 10.1002/ppul.20465

Lamson, D., Rometn, N., Kaprow, V., Lau, Z., Palacios, G., He, L., et al. (2006). Mac2gq polynucleotides- chain reaction-detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. J. Infect. Dis. 191, 1898–1902. doi: 10.1086/508351

Lunek, G., Pagola, E., Ross, P., Amato, B., Possické, L., Cam- panari, G., et al. (2008). Comparison of human metapneumovirus geno- types from the province of Bolzano in Italy with those circulating in northern Italy, and this surrounding region in Italy and Austria. Jpn. J. Infect. Dis. 61, 154–156

Lauritsch, H., Dodman, D., Wat- ton, J. M., and Zamboni, M. 54, 175–180. doi: 10.1111/j.1399-5426.2005.00375.x

“fmich-04-00278” – 2013/9/12 – 11:40 – page 7 – #7
Molecular epidemiology of virus-induced asthma

Mackay, I. M. (2007). Human
rhinovirus 3 infections in
adults induce mild exac-
terbation of asthma associated with increased sputum concentrations of cysteinyl leukotrienes. For: Arach. Mol. Biochem. 138, 267–272.

Mackay, I. M., Bialasiewicz, S., Jacob, K. (2005). Rhinovirus-induced PBMC responses and out-
come of experimental infection in allergic subjects. J. Allergy Clin. Immunol. 105, 692–698. doi:10.1016/j.jaci.2004.10.023

Mackay, I. M. (2006). Genetic diversity of human metapneumovirus over 4 consecutive years in Australia. J. Infect. Dis. 193, 1630–1631. doi:10.1086/507400

Mafi, S. A., Ludevid, H., Kenmoe, L., van Wijk, N., Const, C., and Klompmak, K. F. (2007). Season-
ality, incidence, and repeat human metapneumovirus lower respiratory tract infections in an area with a high presen-
tence of parainfluenza virus type-1 infections. Pediatr. Infect. Dis. J. 26, 693–699. doi:10.1097/01.pid.0000261806.21192

Marohl, C., Espinoza, S., Aho, S. L., Hart, F., and Pethar, P. (2007). Epi-
demiological and clinical features of HRV-BV and RVs in infants in young children. J. Virol. 81, 232–226. doi:10.1128/jvi.02858-06

McPherson, J. W., Rick, E. F., Gobet, C., Grimwood, K., Huang, Q. J., Peeling, D., et al. (2006). Distinct patterns of evolution between respiratory syncytial virus subgroups A and B from New Zealand isolates collected over thirty-seven years. J. Med. Virol. 78, 1594–1598. doi:10.1002/jmv.20272

Menzie, H., Kondo, Y., Sasak, S., Nakata, H., Fukushina, C., Mineta, Y., et al. (2005). Naturally occur-
ning parainfluenza virus 3 infection in adults induces mild exac-
terbation of asthma associated with increased sputum concentrations of cysteinyl leukotrienes. For: Arch. Mol. Biochem. 138, 267–272.

Michalke, P., Shacklelon, L. A., Lamb,
Scott, R., Nissen, M. D., Skov, T., and Mackay, I. M. (2007). Characterization of a newly identi-
fied human rhinovirus, HRV-QPM, discovered in infants with bronchi-
olitis. J. Gen. Virol. 88, 67–75. doi:10.1099/jgv.0.090163-0

Miglioretti, C. L., McWilliam, E. C., Soivalainen-Kepera, C., Hovi, T., and Simmonds, P. (2010). Analysis of the genetic diversity of human rhinovirus species in human rhinovirus species A (HRV-A) and B1 subgroup A virus isolated in Japan in 2007. J. Med. Virol. 82, 526–537.

Nicholso, K. G., Kent, C., and Ireland, D. C. (1993). Respiratory virus infection and exacerbation of asthma in young children. J. Pediatr. 123, 211–215. doi:10.1016/0022-3476(93)90351-A

O’Connell, M. A., McVey, D.,其他人于2007年11月21日，第115卷第11号，第698–705页。
young children (n=100) during a 7- year study in Detroit, France. J. Med. Virol. 82, 1782–1789. doi: 10.1002/jmv.21884

Rahibio-Schon, C., Broc, M., He, J., Khaj, S., Matsumoto, M., Beck, E. T., et al. (2011). Whole genome sequencing and evolutionary analysis of human respiratory syncytial virus A and B from Mombasa, UK. 1998-2010. PLoS ONE 6:e25466. doi: 10.1371/journal.pone.0025466

Rac, G., Jowett, P. H., Thompson, J., Teilliez, S., and Wright, P. F. (1997). Epidemiology and clinical impact of parainfluenza virus infections in ethni- cally healthy infants and young children <5 years old. J. Infect. Dis. 175, 807–815. doi: 10.1086/315397

Richard, N., Komarnitski-Pradel, F., Jouvouehy, E., Mirti, R., Javouhey, E., Aupetit, B., and Gagnadoux, A., et al. (2008). The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr. Infect. Dis. J. 27, 1–5. doi: 10.1097/INF.0b0133-200804935

Robinson, J. L., Lee, B. E., Baston, N., and Li, Y. (2003). Seasonality and clinical features of human metapneumovirus infections in children in Northern Alberta. J. Med. Virol. 76, 98–105. doi: 10.1002/jmv.20239

Rueda, P., Delgado, T., Portella, A., Mokolé, J. A., and Garcia-Barreno, B. (1994). Premature infants colonized with the G glycoprotein of human respiratory syncytial virus resist neutral- ization by monoclonal antibodies. J. Virol. 65, 3734–3739.

Sawicki, A. M., Frankel, S., Malaker, M. N., and Hovi, T. (2002). Genetic clustering of all 102 human rhinovirus prototype strain serotype 87 is close to human enterovirus 70. J. Gen. Virol. 83, 297–305.

Schildgen, O., van den Hoogen, B., and van Doornum, G. J., Fockens, J. C., Cornelissen, J. G., and van Hall, C. B. (1995). Antigenic and genetic variability of human metapneumoviruses. Emerg. Infect. Dis. 1, 686–688. doi: 10.3201/eid0105.940935

Scully, V. N., van den Hoogen, B., and van Doornum, G. J., Fockens, J. C., Cornelissen, J. G., and van Hall, C. B. (1995). Antigenic and genetic variability of human metapneumoviruses. Emerg. Infect. Dis. 1, 686–688. doi: 10.3201/eid0105.940935

“fmicb-004278” — 2013/9/12 — 11:40 — page 9 — #9
stologic role in acute asthma exacerbations requiring hospitalization in adults. J. Infect. Dis. 192, 1149–1153. doi: 10.1086/444592
Wisdom, A., Kulkowska, A. E., McWilliam Latch, E. C., Gaunt, E., Tempelton, K., Harvala, H., et al. (2009). Genetics, recombination and clinical features of human rhinovirus species C (HRV-C) infections; interactions of HRV-C with other respiratory viruses. PLoS ONE 4:e49318. doi: 10.1371/journal.pone.00049318
Volk, D. G., Greenberg, D., Kalkstein, D., Shemer-Avni, Y., Givon-Lavi, N., Saleh, N., et al. (2006). Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. Pediatr. Infect. Dis. J. 25, 320–324. doi: 10.1097/01.inf.0000207395.80657.cf
World Health Organization. (2012). Burden of Disease in DALYs by Sex and Mortality Stratum in WHO Regions, Estimates for 2001. The World Health Report. Geneva: World Health Organization, 192–197.
Woo, M., Sank, M., Seja, J., Olachowicz, H., Buso, W. W., and Stecklik, A. (2008). The presence of rhinovirus in lower airways of patients with bronchial asthma. Am. J. Respir. Crit. Care Med. 177, 1082–1089. doi: 10.1164/rcrm.2007-0975OC
Xiao, N. G., Xie, Z. P., Zhang, B., Yuan, X. H., Song, J. R., Gao, H. C., et al. (2010). Prevalence and clinical and molecular characterization of human metapneumovirus in children with acute respiratory infection in China. PLoS ONE 5, e131–134. doi: 10.1097/EFS.0b013e3181b6a30
Yang, C. F., Wang, C. K., Tellefsen, S. J., Pirastu, R., Lantos, L. D., Chu, M., et al. (2009). Genetic diversity and evolution of human metapneumovirus fusion protein over twenty years. Virol. J. 6, 138. doi: 10.1186/1743-428X-6-138
Yount, K. L., Holman, B. C., Steinmetz, C. A., Eller, F. V., Mizumara, I., Forbus, S., et al. (2007). Severe bronchiolitis and respiratory syncytial virus among young children in Hawaii. Pediatr. Infect. Dis. J. 26, 1081–1086. doi: 10.1097/INF.0b013e318135d3d2
Yoshida, A., Kiyota, N., Kohbayashi, M., Mihmura, K., Tsutui, R., Tsukagoshi, H., et al. (2012). Molecular epidemiology of the attachment glycoprotein (G) gene in respiratory syncytial virus in children with acute respiratory infection in Japan in 2009/2010. J. Med. Microbiol. 61, 825–829. doi: 10.1099/jmm.0.041337-0
Zhang, Y., Xu, H., Shen, K., Xie, Z., Sun, L., Lu, Q., et al. (2007). Genetic variability of group A and B respiratory syncytial virus strains isolated from 3 provinces in China. Arch. Virol. 152, 1427–1434. doi: 10.1007/s00705-007-0884-3
Zlateva, K. T., Lemey, P., Vandamme, A. M., and van Ranst, M. (2004). Molecular evolution and circulation patterns of human respiratory syncytial virus subgroup A: positively selected sites in the attachment glycoprotein. J. Virol. 78, 4675–4685. doi: 10.1128/JVI.78.9.4675-4685.2004
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