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Mini review

First International Symposium on Influenza and Other Respiratory Viruses: summary and overview Kapalua, Maui, Hawaii, December 4–6, 1998

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1. Introduction

This article provides a summary of the information presented at the International Symposium on Influenza and Other Respiratory Viruses organized and convened by The Macrae Group (New York City, NY) in Maui, Hawaii on December 4–6, 1998. This symposium was co-sponsored by the International Society for Antiviral Research and the National Institute of Allergy and Infectious Diseases. The purpose of the symposium was to bring together the leading experts to review the status of the current research regarding respiratory viruses and to provide new insights about the epidemiology, clinical impact, detection including rapid diagnostics, pathogenesis and management of these infections. The emphasis was on novel approaches to immunization and antiviral treatment. The meeting was intended to benefit all those working in the field through a multidisciplinary approach and serve as a forum for open discussion among representatives from academia, government and industry in directing future research.

The meeting was chaired by Frederick G. Hayden, University of Virginia, USA and W. Paul Glezen, Baylor College of Medicine, USA. It was attended by 148 participants from 17 countries. The invited speakers included James Antczak, Trimeris, USA, Curtis Carlson, Biostar

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Inc., USA, Margaret Chan, Department of Health, Hong Kong, Robert Couch, Baylor College of Medicine, USA, Nancy Cox, Centers for Disease Control and Prevention, USA, Janet Englund, University of Chicago, USA, Paul Glezen, Johanna Griffin, Viropharma Inc., USA, William Gruber, Vanderbilt University, USA, Larisa Gubareva, University of Virginia, USA, Frederick G. Hayden, Kelly Henrickson, Medical College of Wisconsin, USA, Robert Hudak, Quidel, USA, Robert Hudson, ZymeTx, USA, Sebastian Johnston, Southampton General Hospital, UK, Paul Mendelman, Aviron, USA, Brian Murphy, National Institutes of Health, USA, Karl Nicholson, Leicester Royal Infirmary, UK, Albert Osterhaus, Erasmus Universiteit, The Netherlands, Amy Patick, Agouron Pharmaceuticals Inc., USA, Anne Pitkaranta, University of Helsinki, Finland, Margo Schilling, Eastern Virginia Medical School, USA, Howard Six, Pasteur Merieux Connaught, USA, Scott Thaler, Merck Research Laboratories, USA, John Treanor, University of Rochester, USA, Ed Walsh, Rochester General Hospital, USA, Estelle Whimbey, M.D. Anderson Hospital, USA, Robert Webster, St. Jude Children's Hospital, USA, Bethanie Wilkinson, Protein Sciences Corporation, USA, and Peter Wright, Vanderbilt University, USA. There were also seven late breaker presentations and a poster session.

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There will be a Second International Symposium on December 10–12, 1999 in Grand Cayman, the Cayman Islands.

2. Epidemiology and impact of respiratory viruses

2.1. International patterns

Respiratory viral infections continue to be leading causes of morbidity, mortality, and economic loss throughout the world. During the last few decades the reported incidence of acute respiratory tract infections has increased globally, and the annual rate of pneumonia has increased in persons ≥ 65 years of age (Han et al., 1999). Some years ago, investigators of ten countries around the world have estimated the global burden of respiratory infections in children (Selwyn, 1990). The incidence of acute respiratory infections among children of 0–59 months of age ranged from 13 to 17 new episodes per 100 child-weeks at risk. The rate of lower respiratory tract infection ranged from 0.2 to 3.4 new episodes per 100 child-weeks at risk. Children spent from 22 to 40% of observed weeks with an acute respiratory tract illness and from 1 to 14% of observed weeks with a lower respiratory tract illness. Case fatality rates varied from 3 to 16% in hospitalized children; respiratory syncytial virus (RSV) was the main pathogen identified. This observation, and other studies, show that in developing countries lower respiratory tract infections in children are leading causes of disability and death (Pelletier et al., 1995; Paxton et al., 1996). However, the pattern of respiratory viral infections depends on geographical location, season, syndrome (upper versus lower respiratory tract), age, and setting (ambulatory versus hospital).

In 312 Filipino children of < 5 years old with an acute lower respiratory infection, 41% were admitted to the hospital and a viral infection was confirmed in 52% (Ruutu et al., 1990). Measles and influenza virus were the most common etiologies identified, and measles and RSV were the leading causes of hospitalization. In Singapore respiratory viral outbreaks among hospitalized infants were associated with RSV infections in 72% of cases and with influenza or parainfluenza viruses in 11% of cases (Chew et al., 1998). RSV outbreaks occurred mainly during the dry season. However, the seasonality of RSV infections differs in other parts of the world; for example, in The Gambia RSV outbreaks occurred during the rainy season (Weber et al., 1998). In Kenya, respiratory viruses were demonstrated in 54% of 822 children with acute respiratory infections (Hazlett et al., 1988). Measles, RSV, parainfluenza viruses (PIV) and adenoviruses were observed, but rhinoviruses, herpes simplex and enterovirus were also associ-

ated with some cases of severe acute respiratory infections. In 328 Indian children under 5 years of age hospitalized with acute respiratory tract infections, 35% were diagnosed with acute bronchiolitis. Viruses were found in 81% of them, and RSV was responsible for 57% of cases of bronchiolitis. Parainfluenza viruses were the next most common, responsible for 11% of cases (Cherian et al., 1990). In Brazil the cause of acute respiratory tract illness was investigated in 175 young children living in the community during a 2-year period (Arruda et al., 1991). Respiratory viruses were isolated from 35% of samples. Of the isolates, 46% were rhinoviruses, 16% PIV, 16% enteroviruses, 10% adenoviruses, 7% herpes viruses and 6% influenza viruses. In this study RSV was not detected; in another study from Brazil, RSV was responsible for 38% of cases of acute respiratory infections in hospitalized children (Sutmoller et al., 1995).

2.2. United States

In the United States the National Health Interview Survey for 1995 estimated that the annual number of acute respiratory conditions in all ages groups was 223 037 000, including 5 113 000 cases of pneumonia and 108 009 000 cases with influenza-like illness (Vital and Health Statistics, 1998). Over one-half of these conditions were medically attended. In children of <5 years of age and in children of 5–17 years of age, the annual number of all respiratory conditions were 32 333 000 and 61 875 000, respectively. The proportion of respiratory conditions medically attended was 80% for children <5 years of age and 47% for children of 5–17 years of age. The annual number of school days lost for an acute respiratory condition is estimated to be about 96 612 000. The number of work-loss days for currently employed adults of 18 years of age or over is estimated to be 134 676 000. Demographic and social factors play an important role in the occurrence of respiratory viral diseases. For example, the impact of these respiratory tract infections is higher in low-income families and in populations with poor social conditions. The rate of documented RSV hospitalizations seems to have doubled during the last 5 years compared to similar surveys carried out in 1977–1983, probably because of wider application of rapid diagnostic assays. During a prospective evaluation of US children hospitalized with an acute respiratory illness between 1991 and 1995, RSV was recovered in 27% of cases, followed by parainfluenza viruses, and influenza (P. Glezen, personal communication). Investigations were negative in 38% of cases.

In the elderly, the rate of hospitalization for pneumonia has increased by 50% between 1985 and 1995. In 1995 it was estimated that 12 262 000 of adults of 65 years of age or over suffered from acute respiratory conditions, which were diagnosed clinically as influenza in 4 401 000 and pneumonia in 684 000. In the elderly 64% of these acute respiratory conditions were medically attended. Despite an influenza vaccination rate of 65% among the ≥ 65-year-old persons, two successive influenza epidemics with excess mortality occurred during the last 2 years. These epidemics are estimated to have resulted in more than 40 000 excess deaths per year. Such observations show that respiratory tract infections are a major public health concern among all age groups and in developed as well as developing countries.

2.3. Molecular epidemiology

2.3.1. The origin of influenza viruses

Molecular epidemiology has yielded insights that are critical to our understanding of how novel human influenza viruses emerge from the virus gene pool present among lower animals and of how influenza viruses are able to evade host immune defenses. The application of genomics has established some of the fundamental properties of influenza viruses in their natural reservoir; aquatic birds are the primordial source of influenza A viruses, there are a limited number of host specific lineages, there is geographical separation and influenza viruses in aquatic birds are in evolutionary stasis but after transfer to other species there is rapid evolution.

Currently there are 15 different influenza A HA subtypes (H1–H15) and nine different NA subtypes (N1–N9) recognized in nature. The *HA*

gene is a key factor in pathogenicity for avian viruses of the H5 and H7 subtypes. The outbreak in Mexico in 1995, which killed thousands of chickens, was caused by an H5N2 strain. This virus acquired pathogenicity because the HA had a polybasic amino acid sequence that could be cleaved by host cell enzymes; highly cleavable HA also occurred in chicken H5 isolates during the Hong Kong outbreak in 1997. Before poultry slaughter, 14% of chicken and 2% of ducks carried H5N1 virus. Molecular analysis of Hong Kong H5N1 viruses revealed that each of the gene segments evolved rapidly except the *HA* gene, suggesting that this gene has been present in domestic avian species for some time. Analysis of viruses isolated from domestic poultry during the Hong Kong outbreak revealed that in addition to H5N1 influenza viruses, a number of other influenza subtypes including H3N8, H6N1, H6N9, H9N2, and H1N9 cocirculated in birds. Internal gene sequence homologies of 97–98% between Quail/Hong Kong/G9/97 (H9N2) and A/Hong Kong/156/97 (H5N1) suggest that an H9N2 virus could have been the source of internal genes of the pathogenic H5N1 virus in Hong Kong. Some evidence suggests that the *HA* gene of the H5N1 virus in Hong Kong may have been derived from an influenza virus of geese (Section 3).

Genetic studies of viral RNA recovered from lungs samples of soldiers that died in 1918 indicate that the 1918 pandemic virus, which killed more than 20 million persons worldwide, was most similar to classical H1N1 subtype swine viruses (Taubenberger et al., 1918). This virus did not possess the polybasic amino acid sequence connecting HA1 and HA2 subunits of the hemagglutinin that is associated with high pathogenicity of some avian influenza viruses of H5 and H7 subtypes. Samples have recently been obtained from humans buried in permafrost in Norway and studies are underway to determine if viral RNA is present. Additional sequence information from different times during the different waves of the pandemic and different parts of the world are required to provide understanding of the remarkable pathogenicity of the 1918 virus.

2.3.2. Surveillance of influenza

An effective surveillance system for new epidemic and pandemic strains of influenza is essential in order to provide early warning of the spread of these variants. Molecular epidemiology is an essential and powerful tool for characterizing these viruses.

Detailed antigenic and genotypic analyses have helped determine the evolution of recent human influenza viruses. In September 1995, an influenza A (H1N1) antigenic variant, represented by A/Beijing/262/95, was identified in China. Antigenic analysis revealed that this virus was distinct from H1N1 viruses circulating during the previous years. Moreover, this virus had a deletion of three nucleotides in the *HA* gene, and this genetic change conferred a dramatic change in antigenicity. By November 1998, this H1N1 variant was detected in patients from Asia, Africa, Europe and North America, and consequently was included in the 1998–1999 influenza vaccine.

Recent analysis of the HA of influenza A/Sydney/05/97(H3N2) virus found that there was a 13 amino acid difference between this virus and the previous H3N2 subtype viruses, A/Wuhan/359/95 or A/Nanchang/933/95. Using these markers it was possible to demonstrate that A/Sydney/05/97 did not originate in Australia and that this virus caused epidemics in Japan in January 1997 and in Korea in February 1997 before appearing in Australia and subsequently spreading to the northern hemisphere.

Since 1987 two antigenically and genetically distinct lineages of influenza B have circulated. These two influenza B virus strains are related to either B/Yamagata/2/87 or B/Yamagata/16/88. Viruses related to B/Yamagata/16/88 have circulated worldwide from 1990 to the present, and a current derivative is included in recent vaccines. B/Yamagata/2/87-like viruses have been detected only in Asia. Surveillance data have shown that this strain was predominant in China during the last 2 years and that outbreaks have occurred in pediatric populations in China. Moreover, recently this strain was identified in other Asian countries. The lack of preexisting immunity for this virus in western populations leaves many individuals susceptible to infection, and concern

about the potential for global spread of this second lineage has increased.

2.3.3. Other respiratory virus infections

New and improved techniques in molecular biology have enabled the development of better diagnostic tools and enhanced our understanding of respiratory viral epidemiology. For example, a multiplex reverse-transcriptase polymerase chain reaction enzyme hybridization assay (RT-PCR-EHA) was used to study epidemiology of six common respiratory viruses. This assay (Hexaplex®) detects influenza A and B, RSV and human PIV types 1, 2 and 3. Previous studies reported that the sensitivity for this assay was 97–100% compared to cell culture isolation (Fan et al., 1998), the specificity being between 95 and 100%. The published positive and negative predictive values are approximately 80 and 100%, respectively.

Samples from children admitted to the Children's Hospital of Wisconsin, Milwaukee, between November 1996 and October 1998 with signs or symptoms of a respiratory tract infection were tested with this multiplex RT-PCR-EHA assay. Most respiratory samples were nasopharyngeal swabs and, to a lesser extent, nasal washes and bronchoalveolar lavages. Approximately 66% of the children tested were moderately to seriously ill with acute or chronic disease, and 25% were immunocompromised. Of 1680 assays, 31% yielded a positive result, and 10% of children had evidence of a dual viral infection. 93% of the viruses were found during a 9-month period from October through June. Among those who were virus positive, 57% of infants less than 3 month of age were RSV positive, 27% had influenza, and 16% PIV. In the 3–6-month age group, 44% were RSV positive, and about equal numbers of PIV and influenza made up the rest. Between 1 and 3 years of age influenza and RSV were the leading pathogens. In those of 6–19 years of age, influenza caused 46% of the hospitalizations and PIV approximately 30%. Pneumonia episodes were equally divided between three viruses: influenza, RSV and PIV. In immunocompromised hosts 40% of the cases identified were due to PIV infection.

The use of molecular diagnostic methods shows that the epidemiology of respiratory viruses is more complex and the impact of these viruses greater than previously recognized.

2.4. Immunocompromised hosts

Discordant results have been reported on the epidemiology of viral respiratory diseases in immunocompromised hosts. In published series many differences exist in types of sample, case definitions, comorbidities, protective measures, immunization status, season of the study and the types of diagnostic assays. For example, in neutropenic patients with pneumonia in the late 1980s and early 1990s, the etiology of pneumonia was considered unknown in 90% of cases. More recent studies indicate that 20–30% of BMT recipients with an acute respiratory illness are infected with a community respiratory virus. About one-half of cases are associated with pneumonia which is associated with a mortality rate of approximately 50% depending on virus type. In 133 consecutive cases of patients with BMT developing a viral respiratory infection, the mortality rates were 61% for RSV, 39% for PIV, 43% for influenza A and B viruses, 72% for adenovirus, 79% for CMV and 100% in seven cases of rhinovirus infection (E. Whimbley, personal communication). Neutropenia secondary to chemotherapy for acute leukemia is also a risk factor for severe community respiratory virus infection, particularly for RSV. An upper respiratory tract infection, such as an acute rhino-pharyngitis, leads to a pneumonia in 60–80% of cases when a myelosuppression is present. These observations justify delaying chemotherapy or transplantation when a patient is suffering from a runny nose or active respiratory tract symptoms.

2.5. Upper respiratory tract complications

Common upper respiratory tract complications of respiratory virus infections include acute otitis media, otitis media with effusion, and acute sinusitis. The main etiologic or predisposing agents are the human rhinovirus, RSV, and human coronaviruses. Adenoviruses, parainfluenza viruses,

influenza viruses and enteroviruses are less commonly documented but important during periods of community activity. Traditionally, the association of viral infection to these syndromes has been documented by virus isolation and serologic methods. Nucleic acid amplification techniques have enabled more frequent detection of human rhinoviruses and coronaviruses and provide new insights into the etiology of sinusitis and otitis media.

Several studies have established a temporal association between respiratory virus infection and otitis media in children. Respiratory viruses have been recovered in middle ear fluid of children with acute otitis media in 5% of cases by culture, in 25% of cases by immunoassay, and up to 53% of cases by RT-PCR. A recent study using RT-PCR found evidence of human rhinovirus, RSV or human coronavirus infection in 75% of children with acute otitis media (Pitkäranta et al., 1998a). The implication of these findings for the medical management of otitis are still uncertain since specific therapy is currently available only for influenza, and it remains to be determined whether early antiviral treatment of acute URI can prevent development of otitis.

Otitis media with effusion is a common childhood disease without a clear etiology although it typically occurs after an episode of acute otitis media. It is estimated that in about three-fourths of cases of acute otitis media in children are followed by otitis media with effusion, which suggest that infection plays a major role. Respiratory viruses have been detected in middle ear effusions by culture in 10% of cases but in up to 30% by RT-PCR (Pitkäranta et al., 1998b). The most frequently recognized one is rhinovirus, although it is unclear whether the detection of viral RNA indicates persistent active replication or residual RNA in trapped secretions.

Radiographic evidences of sinusitis is frequent during natural or experimental common colds, and in reality the common cold is a viral rhino-sinusitis (Gwaltney et al., 1994). Using RT-PCR, viruses, most often rhinoviruses, have been found in up to 40% of sinus samples of adult patients with acute sinusitis (Pitkäranta et al., 1997). Taken together, these studies indicate that human rhinovirus infections are associated with approximately one-half of acute sinusitis in adults and over one-third of otitis media in children.

2.6. Lower respiratory tract complications

Epidemiological surveillance data have established a relationship between morbidity and mortality and the circulation of certain respiratory viruses. Influenza epidemics are a well recognized cause of excess mortality, in particularly the elderly. RSV activity has been linked to both excess hospitalization and mortality in young children but also in the elderly (Nicholson et al., 1997; Han et al., 1999). Among elderly hospitalized with an acute cardiopulmonary event or influenza-like illness, approximately 10% have RSV infection. This frequency was comparable to influenza (11% of cases) among this highly influenza vaccinated population. The death rate among patients hospitalized with RSV was as high as 11% (Falsey et al., 1995). These data suggest that RSV in elderly patients with comorbidities can cause as many hospitalizations and deaths as influenza, when influenza vaccine is widely utilized.

Rhinovirus infection is also an under-recognized pathogen in elderly. In a prospective study of 533 subjects 60–90 years of age with an acute upper respiratory infection, surveillance found 231 virus infections among 211 subjects. Rhinovirus was documented in 52% of virus positive cases, coronaviruses in 26%, influenza A or B in 10% and RSV in 7% (Nicholson et al., 1997). Lower respiratory symptoms complicated 65% of the upper respiratory infections observed in these elderly patients living at home. Among 96 elderly patients with rhinovirus as the sole pathogen, the median duration of illness was 16 days; 61% had lower respiratory tract symptoms and 19% were confined to bed. Lower respiratory tract symptoms were more frequent in patients with comorbidities (Nicholson et al., 1996). These data emphasize that respiratory viruses other than influenza are major causes of infection and morbidity in elderly.

In patients with cystic fibrosis, respiratory viruses are also a major cause of pulmonary complications. In a prospective surveillance study

among children with cystic fibrosis, nasopharyngeal samples were collected at the start of any cold for detection of viral RNA by RT-PCR and for bacterial cultures. During 17 months of observation 38 children suffered 147 colds (Collinson et al., 1996). Picornaviruses were detected in 43% of samples, and 41% were rhinoviruses. Children who experienced more frequent colds had evidence of disease progression judged by clinical score and change in their FEV, as well as more frequent colonization by pathogenic respiratory tract bacteria.

2.7. Asthma exacerbation

Recent studies have shown that respiratory virus infection precipitates acute exacerbations of asthma across all age groups (Nicholson et al., 1993; Gern et al., 1997a; Folkerts et al., 1998) and can lead to hospitalizations and deaths (Johnston et al., 1996). Episodes of acute asthma in schoolchildren were associated with a respiratory virus infection in 80–85% of cases. Of viruses detected, 50% were rhinoviruses (Johnston et al., 1995). Overall children experienced an average of eight colds per year associated with four to five asthma exacerbations. Studies in children and infants admitted to hospital with wheezing illness have produced similar results, with RSV and human rhinovirus together accounting for over 96% of hospital admissions (S.L. Johnston, personal communication). In adult, 44% of asthma exacerbations were associated with virus infections, and the majority were rhinoviruses (Nicholson et al., 1993). Unfortunately, there is currently no antiviral therapy of proven value, and it remains to be determined whether early antiviral therapy of rhinovirus colds might reduce the likelihood of an asthma exacerbation.

3. Influenza A H5N1 virus

3.1. Hong Kong 1997

The ability of influenza viruses to undergo rapid and unpredictable antigenic change has given this virus a prominent place among emerging and reemerging diseases. The occurrence of 18 human cases and six deaths caused by an influenza A H5N1 virus in Hong Kong in 1997 raised concern that next pandemic could be imminent. Based on analysis of poultry samples, it was shown that prior to and at the time of the epidemic, H5N1 viruses were circulating in poultry farms and live bird markets in Hong Kong. Analysis of H5N1 viruses isolated from humans during the outbreak revealed that all eight RNA gene segments of these viruses were of avian origin. The *HA* gene was closely related to the Eurasian lineage of viruses. Antigenic analysis revealed that there were two antigenic groups that possessed corresponding genetic changes encoding a potential glycosylation site at amino acid 156 in only one of the two groups. The NA protein of these viruses shares a deletion of 19 amino acids in the stalk region. As discussed above (Section 2.3), the pathogenic H5N1 recovered in Hong Kong appears to have the internal genes of an avian virus of quail, and perhaps the H5 of avian virus from geese. Epidemiological studies indicated that the transmission was primarily from poultry to humans and was inefficient. Recent experiments with chickens suggest that the transmission of H5N1 among poultry was fecal-oral and probably not by aerosol. Whether this occurred by respiratory droplets or by a fecal-oral route in humans is unclear. Human to human transmission may have occurred in a limited numbers of cases. No new humans cases have been detected since poultry culling took place in December 1997. Nevertheless, concern remains that H5N1 viruses may pose a reemerging threat at some time in the future.

3.2. Pathogenesis

The reasons for the unique virulence of the Hong Kong virus in humans are uncertain. The H5N1 viruses isolated from chickens and humans possessed hemagglutinin molecules with multiple basic amino acids adjacent to the HA cleavage site, a finding characteristic of virulent avian influenza viruses. This observation, and the fact that the H5N1 virus was lethal for chickens are consistent with the presumption that this insertion allows cellular proteases to cleave the

hemagglutinin and enable the virus to spread to visceral sites outside of the respiratory tract. However, extrapulmonary dissemination of virus was not documented in human cases in Hong Kong. When four H5N1 human isolates (HK156, HK483, HK485 and HK486) were inoculated into BALB/c mice, each virus replicated well in mouse lung. These viruses were lethal for mice and replicated in multiple organs but showed different patterns of virulence. HK483 and HK485 were 100% lethal at any dose, HK156 was intermediate, and HK486 was associated with relatively low mortality of 10%. Brain invasion was associated with disease progression and lethality but was not observed in mice infected with the HK486 virus, which replicated only in the lungs. Viral antigens were detected in smooth muscle and in the brain. Thus, strains of the Hong Kong H5N1 virus were competent and in some instances neurotropic without prior adaptation to a mammalian host.

3.3. Vaccine

Experiments have shown that challenge of poultry with H5N1 containing an HA modified by deleting the polybasic amino-acid sequence is safe. Vaccination of poultry with a non-pathogenic H5N3 influenza virus induced antibody against HK156 and HK483 viruses. After challenge with a virulent H5N1 strain, virus titers in lungs were undetectable and no replication occurred in brain in the group that received the H5N3 vaccine.

Another vaccine for the H5N1 strain of influenza was developed utilizing the baculovirus expression vector system (Treanor et al., 1996). The subunit vaccine contains a purified, genetically engineered version of the hemagglutinin protein. Preclinical data demonstrated that the vaccine protected chickens against disease and death following challenge with a lethal dose of the parental virus. In addition, this vaccine was shown to induce cross-protection against A/Hong Kong/438/97 (H5N1) infection. Phase I clinical trials with low doses of the purified H5 subunit vaccine have shown good tolerance. Initial antibody responses were detected in 40–60% of volunteers by ELISA, 85–100% by Western blot assay, but very infrequently by neutralization assays. Dose escalation studies are ongoing.

4. Pathogenesis and immune response

4.1. Influenza virus

The characteristics of immunity to influenza are well known: it exhibits subtype/variant specificity, reduced effectiveness for succeeding naturally occurring virus variants, and is prolonged in duration (well demonstrated with the immunity present with reoccurrence of H1N1 viruses in 1977). Immunity is present early after exposure so that markers for infection remain negative, and is active at the mucosal surface (Couch et al., 1984). Immune mediators that share these characteristics are antibodies to the hemagglutinin or the neuraminidase as either IgG or as IgA in serum or secretions. Anti-hemagglutinin antibody will inhibit adsorption of virus and can contribute to inhibition of release from infected cells, while the major function of antineuraminidase antibody is inhibition of viral release so that spread of infection is impaired (Dowdle et al., 1974). In human studies, infants born of mothers with high levels of neutralizing antibody were protected for longer periods, thus demonstrating the value of passive immunity directed toward the subunits (Puck et al., 1980). An increase in anti-neuraminidase antibody, as a result of vaccination was associated with a reduced amount of virus in nasal secretions, a finding consistent with the concept of neuraminidase activity (Couch et al., 1974). When these various antibodies were quantitatively related to the occurrence of infection and illness, it was noted that serum neutralizing, nasal secretion neutralizing, serum anti-neuraminidase, and nasal secretion neuraminidase antibody all mediated reduced severity of the infection with increasing titers of the antibody (Couch, personal communication). Thus, all antibody modalities appear capable of contributing to immunity. That serum IgG anti-HA antibody is the most important mediator based on studies indicating IgG is the dominant antibody in lower respiratory secretions and it is derived from serum. Since, the majority of infections are considered to be initiated by small particle aerosol with deposition of virus about equally in the nasopharynx and lower respiratory tract and the infectivity for the lower

respiratory tract is 30- to 100-fold lower that that for the nasopharynx, serum IgG antibody to the HA would be the first line defense against infection (Couch et al., 1984).

More recent studies on immunity derive mostly from animal models. In a recent study in a IgA knockout mouse, there were no differences in infection responses between mice with and without IgA antibody in serum and secretions suggesting that this antibody is not required for protection (Mbawuike, unpublished data). Studies of influenza in mice recently have focused on the role of specific lymphocytes and cytokines in immunity. These have confirmed earlier studies that CD8 cytotoxic lymphocytes (CTL) occur in the lower airways and appear to be primary mechanism for recovery from virus infection. These cells control pneumonia via a perforin or FAS-mediated cell destruction mechanism. In knockout mice it was clear that either CD4 or CD8 deficient animals can recover in a near normal fashion. In contrast, when both are absent, there is a marked impairment in recovery from infection (Eichelberger et al., 1991; Scherle et al., 1992; Topham and Doherty, 1998). Cytokine studies have show the dominance of interferon gamma in lungs during infection while a number of other cytokines are also present that could contribute to disease and recovery (Sarawar et al., 1993; Kelso et al., 1996). Overall, the large number of factors described that can contribute to the inflammatory response and to control of virus replication indicate the recovery process is complex.

Studies of lymphocytes in humans have shown that CD8 cells mediate cytotoxicity and that the cell exhibiting this cytotoxicity is an RO+ memory cell (Di et al., 1994) (Mbawuike, personal communication). When the level of cytotoxicity was quantitated for a group of young adults and elderly adults, it was shown that all young adults exhibit significant cytotoxicity whereas its occurrence among elderly individuals was erratic, a finding that might contribute to their susceptibility to complications of influenza (Mbawuike et al., 1997). Difficulty with providing data in humans comparable to inbred mice relates to methodology. Interferon gamma spot forming cells appear to be an acceptable substitute for CTL responses; more recently, MHC peptide-specific tetramers have been used for precise quantitation of CD8 memory cells (McMichael and O'Callaghan, 1998). Further development of this technology should permit human studies of the role of CD8 CTL function in influenza infection and disease.

4.2. Respiratory syncytial virus

4.2.1. Pathogenesis

RSV is a single-stranded, negative-sense enveloped RNA virus which replicates in a variety of cells in vitro. However, in humans, RSV replication appears to be restricted to respiratory epithelial cells. RSV antigens can be detected in ciliated cells but also in the submucosa. RSV fusion protein is responsible for the syncytia formation, which gives the virus its name (Delage et al., 1984) and the G protein has the cell attachment motif. The extent of replication in the small airways is unknown during bronchiolitis in infants and young children.

Bronchiolitis is characterized by atelectasis, hyperinflation and increased airway resistance. The mechanisms leading to narrowing of the airways include unfavorable mechanics of the infant lung, epithelial damage, submucosal edema, and bronchiolar reactivity (Stark et al., 1996). Microscopically, RSV infection of immunocompetent children is associated with epithelial cell injury of bronchioles, desquamation, luminal plugging by mucus and eosinophilic material, and squamous metaplasia. Submucosal edema is often prominent but neutrophils are uncommon. The alveoli are relatively spared and giant cells/syncytia are infrequent. In the immunocompromised host, giant cell pneumonia and syncytia are commonly found along with inflammatory infiltrates containing neutrophils. Epithelial damage produced by RSV appears to be due both direct viral cytopathic effect and other mechanisms such as upregulation of chemotactic factors and production of multiple cytokines (Noah and Becker, 1993; Arnold et al., 1994; Renzi et al., 1997; Smyth et al., 1997).

4.2.2. Immune response

The early age of hospitalization and the observation that viral replication is higher and sustained for 8–10 days during primary infection confirm that primary infection is associated with the greatest risk of severe disease. However, reinfection with RSV occurs episodically throughout life (Henderson et al., 1979). Animals models, mainly cotton rat and mouse, have demonstrated that immunity against the F protein provides a greater protection against reinfection than immunity against the G protein or the internal proteins. In the bovine model, there is a suppressive effect of maternally transferred antibody. In all animals a protective effect of passively transferred antibody can be demonstrated at circulating neutralizing antibody titers of 1:200–1:400. In humans, maternal antibody appears to provide partial protection against serious disease, and RSV-specific immune responses reduce the risk of subsequent infection involving the lower respiratory tract.

Bone marrow and solid organ transplants patients have severe RSV infections, and prolonged RSV shedding is described in HIV-infected infants. Thus cell mediated immunity plays a major role in clearing virus. Depletion of both CD4 and CD8 cells in the mouse leads to prolonged RSV shedding. On the other hand, infusion of a large numbers of RSV-specific CD8-positive, cytotoxic T-cells can cause severe disease in the mouse model of RSV infection. This observation may be relevant to early efforts at protecting infants with a formalin-inactivated vaccine. This vaccine was immunogenic as judged by antibody rises and lymphoproliferative responses to RSV. However, natural exposure to RSV resulted in more severe illness than usual, and a high percentage of children were hospitalized with bronchiolitis and pneumonia at an age where serious illness would not be expected. Eosinophilia was predominant in the airways of the affected children and an aberrant immune response to natural infection because of prior exposure to the formalin inactivated vaccine has been presumed.

Different patterns of pulmonary cytokines responses have been found in mice given different vaccines and then exposed to RSV. In mice immunized with formalin-killed vaccine the response was characterized mainly by IL4 and eosinophils, whereas natural infection was associated with interferon γ in the lungs. Wild-type RSV infection induces generally a Th1 cytokine pattern, that is important in viral clearance, while inactivated or subunit vaccine has shown mainly a Th2 pattern (Jackson and Scott, 1996; Konig et al., 1996; Johnson et al., 1998). Presentation of individual proteins, particularly F and G, in a live vector so that antigen presentation would be predicted to follow a Th1 response does not lead to enhanced disease or Th2 mediated response in rodents. Recent studies have suggested that the unique G protein may stimulate eosinophilia (Johnson et al., 1998). Such observations indicate that immunization against RSV faces important immunologic hurdles in naïve children.

4.3. Parainfluenza virus

There are three major parainfluenza virus (PIV) serotypes, designated types 1, 2 and 3. PIV 3 is endemic, sometimes causing spring epidemics, while PIV type 1 and 2 cause fall epidemics in alternating years. PIV 3 is the most frequently isolated and most likely to cause disease in infants and in immunocompromised hosts. There is a high frequency of reinfection which is usually less severe than the first infection. Evidence of protective immunity is seen in the striking difference in virus replication on live vaccine challenge of immunologically naïve as compared to previously naturally infected children.

Like influenza virus, sialic acid receptors are thought to be the PIV cellular recognition site and PIV fusion protein undergoes cleavage activation. PIV, like RSV, replicates in superficial epithelial cells of the respiratory tract and buds apically from the cell. Antibodies to the two major surface proteins, namely hemagglutinin-neuraminidase protein (HN) and fusion protein (F) confer protection. Data from experimental parainfluenza 1 has shown the importance of mucosal IgA antibody in protection against respiratory infection. Virus-specific IgA during its transcytosis across the epithelial cell may interfere with virus maturation. PIV 1 and 2, which cause croup and laryngitis, probably replicate in the major airways only.

PIV 3 is similar to RSV and causes lower respiratory tract infections. In contrast to RSV infection, it seems unlikely that there is a major immunologically mediated component to PIV illness. Enhanced natural disease was not observed after administration of killed vaccine.

4.4. Rhinovirus

4.4.1. Site of replication

Some respiratory viruses, particularly adenovirus, RSV and influenza are known to infect and replicate in the lower respiratory tract epithelium. In contrast, the ability of rhinoviruses to infect the lower respiratory tract is controversial. Rhinovirus type 16 has been detected by RT-PCR in bronchoalveolar lavage obtained from experimentally infected volunteers; however, contamination of the samples with rhinovirus from the upper airway could not be excluded (Gern et al., 1997b). In-situ hybridization to detect the rhinoviral genomic RNA can discriminate between cellular infection and contamination from the upper respiratory tract by demonstrating the presence of virus within bronchial epithelial cells. In bronchial biopsies from ten experimentally infected subjects (Fraenkel et al., 1995) rhinovirus was detected in the bronchial epithelium of four subjects when samples were taken 4 days post infection (Bates et al., 1998). In contrast no virus was detected in the biopsies taken before the challenge. These data strongly suggest that rhinovirus is capable of replicating in lower airways epithelium. How often this occurs under natural conditions remains to be determined.

4.4.2. Inflammation and airway hyperreactivity

Non-asthmatic persons undergoing a rhinoviral infection will develop principally upper respiratory symptoms and only infrequently exhibit lower respiratory symptoms. During rhinovirus-induced asthma exacerbation, nasal aspirates of children have increased levels of IL-8 and neutrophil myeloperoxydase. Increased nasal wash IL-8 levels also correlate with airway hyperreactivity in asthmatic subjects experimentally infected with rhinovirus type 16 (Grunberg et al., 1997a; Teran et al., 1997). These results suggest that neutrophils are involved in the inflammatory response to rhinovirus, but their role in lower respiratory tract infection is unclear. Eosinophil major basic protein is increased in nasal secretions during rhinovirus infection in asthmatic children, and increased levels of the eosinophil cationic protein have also been observed in sputum in asthmatic subjects undergoing experimental rhinovirus infections (Grunberg et al., 1997b). Eosinophils are found in increased numbers in the airways of asthmatic persons with experimental rhinovirus infection, which suggests that eosinophil infiltration is a crucial element of the pathology leading to exacerbation of asthma (Fraenkel et al., 1995). In addition, $CD3^+$, $CD4^+$ and $CD8^+$ lymphocyte infiltration occurs in the lower but not in the upper respiratory tract epithelium and submucosa during experimental rhinovirus colds. In vivo studies have demonstrated that HRV infection of respiratory epithelial cells induces its own receptor, ICAM-1 (E. Arruda, personal communication). Up regulation of ICAM-1 and VCAM-1 by HRV infection may play a role in the retention and activation of intraepithelial lymphocytes and eosinophils. As VCAM-1 is important in eosinophil infiltration, factors regulating its expression may therefore represent targets for therapeutic intervention.

The eosinophil infiltrate observed in asthmatic subjects during viral infection may also result from epithelial cell production of cytokines/chemokines with direct actions on eosinophil recruitment and activation. RANTES levels are increased in nasal aspirates from children with virus-induced exacerbation of asthma and in HRV-infected bronchial epithelial cells under in-vitro conditions (Johnston, 1997). These findings need to be confirmed and their relative importance in asthmatic and normal subjects investigated.

The degree of redundancy among cytokine functions suggests that even if one important molecule's functions could be blocked, other molecules may fulfill a similar role, and render a treatment aimed at one specific molecule ineffective (Johnston, 1997).

5. Influenza diagnosis

5.1. Influenza RT-PCR

Molecular virologic techniques have also been applied for enhanced diagnosis of influenza virus infection. During the influenza season of 1997–1998, RT-PCR for influenza virus was compared to serology and culture in 966 patients with influenza-like illness in 12 countries. Samples for culture and RT-PCR were combined nose and throat swabs. Overall 54% of cases were culture positive, 65% showed fourfold or greater antibody titer rise and 70% were RT-PCR positive. Only 43% of patients were positive by all three tests and 9% of patients were positive by a single test only: RT-PCR only in 42, serology only in 41 and culture only in eight. Virus typing and subtyping information was 100% concordant between all the tests. These data show that RT-PCR is a reasonably rapid, highly reliable test for detection of influenza and is capable of enhancing the sensitivity for detecting influenza.

5.2. Rapid assays

5.2.1. Immunoassays

Several rapid assays for influenza A and B virus detection are available or in development. At this time, data comparing the sensitivity and specificity of these tests with each other are lacking. Moreover, the utility of these tests in elderly and other high-risk population has not been critically determined. Before reaching final conclusions concerning the utility of these tests in clinical practice and their role in guiding antiviral therapy, further studies are necessary.

In the United States three commercially available rapid diagnostic tests are approved. One of these, Directigen® Flu A, is a colorimetric immunoassay that detects the nucleoprotein of influenza A viruses. Another one is an optical immunoassay, based on the properties of light reflection and thin film amplification. This assay allows detection of the nucleoproteins of influenza A and B viruses (Influenza OIA, BioStar®). This assay was tested during the 1997–1998 season in 404 specimens obtained from a group of 184 patients with fever and influenza-like symptoms of less than 36-h duration. Patients were recruited in the community and ranged in age from 2 months to 76 years. The type and numbers of specimens varied for each patient with an average of 2.5 specimens collected per patient. Virus isolation in cell culture was performed on each available specimen. When compared to culture, the sensitivity of the rapid assay varied from 52% for throat swabs, 65% for nasopharyngeal swabs, 80% for nasal aspirates and 90% for sputum. When patients with a positive culture in any type of sample were considered as having proven influenza, the assay had a relative sensitivity by specimen type of 50% for throat swabs, 68% for nasopharyngeal swabs, 76% for sputum, and 80% for nasal aspirates.

An investigational sandwich immunoassay using monoclonal antibodies directed against type-specific nucleoproteins of influenza A and B viruses has been developed recently (Gold-Labeled-Optical-Readout-Assay®, Roche Laboratories, Germany). The performance of this test was compared to virus isolation on MDCK cells during the 1997–1998 season. Samples were throat swabs performed in 158 children with influenza-like illness. The sensitivity of the test compared to culture was 85–90% and the specificity 81–93%. All culture negative but Gold-Labeled-Optical-Readout-Assay® positive tests were PCR positive for influenza RNA, an observation suggesting enhanced sensitivity. Preliminary results with this test are encouraging but further studies in adults and elderly are necessary.

Other new rapid prototype tests using sandwich assay technology targeting conserved epitopes present early during infection are under development. Other targets could be the M1 protein which is a internal membrane protein representing 40% of all virions protein. However, this membrane protein is difficult to extract in aqueous solution so that most assays have targeted the more readily extractable, type-specific nucleoproteins.

5.2.2. Neuraminidase based assay

Influenza A and B viruses possess surface glycoproteins with neuraminidase activity that-

hydrolyzes substrates containing alpha-ketosiadically linked *N*-acetylneuraminic acid (Neu5Ac). A modified Neu5Ac molecule coupled with a chromogen was designed to produce a novel neuraminidase substrate selected to fit only in the influenza neuraminidase active site. After the chromogen portion is cleaved by neuraminidase enzymatic activity, its precipitation yields a blue color. Extraction of the virus, addition of reagents and incubation are done in about 1 h. Clinical studies done during the 1995–1996 season using throat swabs on 157 patients revealed a specificity of 99% and a sensitivity of 65% compared to culture. The test has been modified to improve sensitivity and shorten the procedure to 30 min. However, this new assay has not been tested in large numbers of clinical samples, and results with other specimens such as nasal wash are not yet available.

6. Vaccines and prevention strategies

6.1. Influenza viruses

6.1.1. Strategy to improve the immune response

Although they are relatively inexpensive and increasingly utilized, inactivated influenza vaccines suffer from a number of disadvantages. In particular, they induce responses with limited durability and induce very limited cross-strain protection, are contraindicated for individuals with severe egg allergy, and are less effective in frail older adults, in whom the complications of influenza are most severe. One strategy to improve the immune response is the use of adjuvant. ISCOM (immunostimulating complexes) are cage-like structures containing different exogenous non-replicating antigens. The ISCOM presentation system induces CD8 CTL response and can induce antibodies even in the presence of neutralizing antibodies. ISCOM influenza vaccine studies in macaques have been shown to be superior to classical vaccine in term of antibody and T-cell responses and protection. An ISCOM vaccine containing H5N1 virus provides superior antibody responses in chickens, but no data are currently available on protection. Preliminary clinical studies using different ISCOM preparations have failed to demonstrate any clear benefit in humans and the development has been abandoned at present.

Cold-adapted influenza vaccine given intranasally has multiple theoretical advantages: induction of a mucosal immunity, induction of a cytotoxic lymphocyte response, protection of the upper respiratory tract, and broader and perhaps more durable immune responses. In addition, an intranasal vaccine is easier to administer than intramuscular injection. New antigenic variants can be rapidly produced using genetic reassortment between a cold-adapted donor strain and a wild type virus. The cold-adapted influenza vaccine, using A/Ann Arbor/6/60 and B/Ann Arbor/1/66 viruses are reproducibly infectious, attenuated, not transmissible, genetically stable after prolonged shedding, and induce protective immunity in children and adults. In combination with inactivated vaccine, they are reported to increase protection in the elderly.

Placebo-controlled phase III trials have been conducted to assess efficacy of trivalent cold-adapted vaccine (CAIV-T, FluMist™) in children, given in one dose or two doses, against culture-confirmed influenza during two consecutive seasons, 1996–1997 and 1997–1998. Vaccine was modified during the second season (to be adapted to the H1N1 circulating strain) but contained an H3N2 virus that was different from the predominant circulating A/Sydney/97 (H3N2) virus. During the 1st year 1070 children were enrolled in the vaccine group and 532 in the placebo group. Most (87%) children received two doses during the 1st year. Among the children seronegative at baseline the percentage of those showing a positive HAI antibody response, defined as a fourfold or greater increase in titer, ranged between 16 and 92% for each of the three vaccine strains after the first dose and between 61 and 96% after the second dose. This vaccine was generally well tolerated but associated with a slight excess of rhinorrhea.

During the 1st year the overall efficacy was 93% against culture-confirmed influenza (Belshe et al., 1998). The frequency of confirmed influenza during the 1st year was 18% (12% influenza A and

7% influenza B) among the placebo recipients and 1.3% in the vaccine group (0.7% influenza A and 0.7% influenza B). Among vaccinees the severity of culture-positive influenza illness was markedly attenuated. During the 2nd year, a new drift variant appeared after production of the vaccine and caused all but four cases of influenza illness. The CAIV-T provided a protective efficacy of the vaccine of 100% against illness due to influenza A (H3N2) Wuhan-like virus and 86% against Sydney-like virus. The results show that the vaccine provided solid protection against both homologous wild type virus and against a drift variant not represented in the vaccine. In addition, this vaccine provides protection against influenza associated otitis media and lower respiratory tract disease.

A placebo-controlled study of CAIV-T was also conducted last season in over 4500 healthy working adults. The end-points included incidence of influenza-like illness (defined as 2 days with at least two influenza-related symptoms), severe influenza-like illness (3 days of symptoms), upper respiratory tract infection, and febrile upper respiratory tract infection. During the 14-week outbreak period, the CAIV-T vaccine significantly reduced days of severe influenza-like illness by 14% and days of febrile upper respiratory tract infections by 12%. The number of overall days of missed work was reduced by 16% for influenza-like illness (5.7 days/100 subjects). This trial did not attempt to document the etiology of illness in affected persons.

6.1.2. DNA immunization

DNA vaccines are highly purified bacterial plasmid DNA vectors containing a promoter element driving the transcription and translation of the coding sequence for the antigen of interest. Although the precise mechanism of action remains controversial, it is generally accepted that the purified DNA is taken up by cells and the antigens are then synthesized inside the cells. This neosynthesis of antigen inside the host cells leads to the association of the antigens with class I or II MHC molecules, migration to the cell surface and presentation to cytotoxic T lymphocytes or T helper cells. In this manner, DNA vaccines are able to elicit immune responses similar to attenuated live viral vaccines. In addition to this novel means of MHC class I antigen presentation, DNA vaccines offer several other advantages. DNA is thermally stable and because the plasmid persists for some time at the site of inoculation, responses may be more durable than those induced with protein vaccines. DNA vaccines are not associated with viral vector-induced immune responses which can dampen immune responses to target antigens. In the context of influenza vaccination, DNA is not produced in eggs and may not require annual re-vaccination. Adjuvants may improve immune responses to DNA vaccines by a number of mechanisms, including improved efficiency of transfection of DNA, improved targeting of DNA to specific cell types, activation or recruitment of antigen presenting cells, or protection of DNA from degradation. In animals, aluminum adjuvant (alum or aluminum phosphate) improves antibody responses but does not increase plasmid protein expression.

Utilizing DNA vaccine technology obtained from Vical Inc., Merck & Co., Inc., has developed an influenza DNA vaccine (IDV) encoding A/Georgia/03/93 (H3N2) hemagglutinin (HA) which is currently in ongoing phase I clinical trials at the Center for Immunization Research, Johns Hopkins School of Public Health. In pre-clinical testing HA DNA vaccination generated hemagglutination inhibition responses (HAI) in all species tested including mice, ferrets, guinea pigs, African Green and rhesus monkeys. Protection following challenge was seen in mice following as little as 1 μg of HA DNA, and 100% protection was seen with a single 10-μg injection in mice. Other genes such as *NP* appear to provide cross-strain protection. Preliminary data from the two ongoing, double-blind, phase I studies have been analyzed. In the first study, IDV was evaluated in 141 adults randomized to receive up to three doses (1–500 μg HA DNA) or placebo. Some subjects were boosted with commercial inactivated influenza vaccine. A second study was conducted in 78 adults who were randomized to receive up to three doses of IDV alone (300 μg DNA) or IDV (100, 300, or 500 μg) with aluminum adjuvant (225–700 μg) or placebo. No

serious adverse events attributable to IDV vaccination or adjuvant were reported. Virus neutralizing (VN) and hemagglutination inhibiting (HAI) antibodies were observed in a minority of subjects after three vaccinations with IDV alone. HAI responses following inactivated vaccine suggested priming by IDV. Modest VN and HAI antibody responses were observed after vaccination with IDV and aluminum adjuvant.

A great deal more work on DNA vaccines is needed. Achieving both robust CTL and antibody responses has been problematic in man. Safety concerns include genomic integration and generation of anti-DNA antibodies. The optimal dose, delivery method and the role of adjuvants are not clear.

6.2. Respiratory syncytial virus

6.2.1. Vaccine development

RSV cpts248/404 is a live-attenuated, cold-passage (cp), temperature-sensitive (ts) vaccine virus. Sequence analysis showed that cold passage and chemical mutagenesis introduced three nucleotide substitutions: two in the L protein and a third in the *M2* gene (404-M2 mutation) (Skiadopoulos et al., 1999). Detailed study found that the 248 mutation in L specifies a significant reduction of plaque formation at 38°C and is responsible for intermediate attenuation in mice. In contrast, the other L mutation did not contribute to the temperature sensitive or the attenuation phenotype. Unexpectedly, the 404-M2 mutation alone specified complete restriction of plaque formation at 37°C and high level of attenuation in mice. Thus the cpts248/404 virus contains a set of temperature sensitive and non-temperature sensitive mutations which account for its genetic stability. A recombinant version of this virus was phenotypically indistinguishable from cpts248/404. It represents a background into which additional mutations can be introduced to obtain the desired level of attenuation.

The current RSV vaccine candidate, cpts248/404, failed to infect 15 seropositive children aged 15–59 months. The vaccine was safe and genetically stable in seronegative children aged 6–24 months at doses of 10^4 or 10^5 pfu (plaque forming units). Two doses were administered (10^4 or 10^5) in 4–12-week-old infants. Based on detectable virus shedding, one dose was infectious in 76–100% of infants. Two isolates post-immunization showed a reduction in their temperature sensitivity phenotype. At either inoculum the vaccine caused mild to moderate nasal congestion but no lower respiratory tract symptoms despite high level of nasopharyngeal replication. After the second inoculation, virus shedding was detected in only four of 22 cases. These preliminary results suggest that protection occurred in absence of detectable serum IgG antibody response. However, the presence of respiratory symptoms suggests that a more attenuated vaccine is necessary for infants. A second derivation of cpts248-404 bearing an additional ts mutation, cpts248/404/1030, is more temperature sensitive and more attenuated than the parent; clinical trials with this virus will be initiated (Whitehead et al., 1999).

6.2.2. RSV immunization in adults

Much of the information necessary to assess the potential value of a RSV vaccine in adults is lacking. Knowledge about protective immunity, epidemiology and appropriate target population is limited. Unlike in infants, rapid antigen tests and culture are insensitive for detecting RSV infections in adults. However increasing evidence suggest that RSV is a major pathogen in elderly adults, particularly in those with underlying cardiopulmonary conditions, and in immunocompromised hosts. Since 1977, 17 outbreaks of RSV infections in nursing home populations have been reported.

Experimental challenge studies in adults suggest that resistance to infection is correlated with the titers of serum and mucosal RSV neutralizing antibody. Observation and case-control studies in elderly adults suggest that the severity of illness is inversely correlated to the level of humoral immunity. Infected elderly had fourfold lower titers of serum neutralizing antibody than controls.

Thus far, three candidate vaccines have been approved for phase I or II testing in elderly adults. These include a purified fusion protein subunit vaccine (PFP), a chimeric subunit F-G (fusion-attachment protein) vaccine, and a live-at-

tenuated, cold passaged temperature-sensitive (cpts) mutant virus. A phase I safety and immunogenicity test of PFP vaccine involved 64 elderly adults. Intramuscular injection was well tolerated, and 57% of recipients had fourfold or greater response to F protein by ELISA and 61% had fourfold or greater neutralizing antibody responses. Subsequent study in frail elderly nursing home residents found antibody responses in 53 and 47% of vaccinees, respectively. In healthy elderly of 70 years of age or older, the responses were 76 and 61%, respectively. Further evaluations with clinical end-points are necessary.

6.2.3. RSV immunoglobulins

Reinfections with RSV are associated with considerable reduction of illness severity (Henderson et al., 1979). Maternal antibody to RSV passively acquired by the fetus has been shown to protect against lower respiratory tract illness. Animal model studies have demonstrated the potential benefit of passively administered RSV immunoglobulins (Prince et al., 1985; Siber et al., 1994). Subsequent trials of humanized murine monoclonal antibodies directed to the F protein have yielded different results. In the cotton rat model protection against RSV lung infection was achieved by passively transferred antibody (Graham et al., 1993). In primates human IVIG administered locally or systematically are effective in reducing titers of virus recovered from lungs and nose. Polyclonal preparations with high titers of neutralizing antibody were shown to provide protection against severe RSV illness in premature infants and children with bronchopulmonary dysplasia (Hemming et al., 1995; The PREVENT Study Group, 1997). One monoclonal antibody preparation, Medi-493 (palivizumab, Synagis™), was shown to be effective in preventing hospitalization for RSV in premature infants and children with bronchopulmonary dysplasia. In a randomized study among 1502 children with prematurity or bronchopulmonary dysplasia, palivizumab resulted in a 50% reduction of hospitalization for RSV (10.6% in the placebo group vs. 4.8% in the palivizumab group). This effect was mainly observed in the subgroup of infants with prematurity (8.1 vs. 1.8%, respectively) and was less significant in children with bronchopulmonary dysplasia alone (12.8 vs. 7.9%, respectively) (The IMpact-RSV Study Group, 1998). Another monoclonal antibody, RSHZ19, did not provide a protective effect, possibly because of lower neutralizing activity. Considering the high cost of these preparations, prophylaxis should be considered only in high-risk patients such as children of less than 2 years of age with chronic lung diseases, and possibly in premature infants of 32 weeks or less during the RSV season.

Although prophylaxis has shown efficacy, treatment with these preparations appears less promising (Hemming et al., 1987). An early trial found that human IVIG treatment appeared to reduce virus shedding and to improve oxygen saturation in young infants hospitalized with RSV infection. However RSVIG containing high titers of neutralizing antibody to RSV reduced pulmonary virus titers but did not improve clinical outcome such as days of hospitalization, use of ICU or need for intubation in hospitalized infants Rodriguez et al., 1997). A recent study, with monoclonal antibody to RSV F protein in intubated children found similar negative results.

These results indicate that treatment with polyclonal or monoclonal RSV antibody preparations in infants with established lower respiratory tract disease does not alter symptoms or overall outcome. As our understanding of bronchiolitis improves, it appears that any strategy that targets virus shedding alone will have limited results. It remains to be determined whether early treatment of upper respiratory tract RSV illness can prevent progression to lower respiratory tract disease.

6.3. Parainfluenza viruses

Human parainfluenza viruses particularly PIV3 are an important cause of hospitalization of infants and children. The attack rate for PIV3 lower respiratory illness is higher in infants younger than 6 months of age. Consequently, prophylaxis should be given early in the life and is effective in the presence of maternal antibodies. Natural infection with PIV does not prevent reinfection but protects against severe disease suggesting that multiple doses of vaccine may be necessary. Sig-

nificant antigenic drift does not occur in PIV3, so that a monovalent vaccine should be protective. Optionally vaccine should stimulate humoral, mucosal and cell-mediated immunity as does natural infection.

A phase 1 clinical trial of a bovine parainfluenza type 3 (bPIV3) live intranasal vaccine was conducted in 96 subjects. Participants included 12 infants, 66 children, and 18 adults, who received 10^3–10^6 $TCID_{50}$ (tissue culture infectious dose 50%) intranasally. The vaccine appeared to be safe. Among 37 seronegative children (6–60 months of age) 85% were infected and 61% of recipients developed a level of antibody considered to be protective. The vaccine conserved its phenotype after replication in vaccinees and was not transmissible. A single dose of the vaccine infected 92% of the infants less than 6 months of age and induced seroconversion to bovine-PIV3 in 67% and to human-PIV3 in 42% of recipients.

A randomized double-blind phase 2 study with different dosages is ongoing: 192 infants of less than 2 months of age have been enrolled and immunized by intranasal sprays at 2, 4 and 6 months with placebo or 10^5 or 10^6 $TCID_{50}$. Side-effects, mainly respiratory complaints were observed in 20–40% of patients in the combined study groups. Serum hemagglutination inhibition (HAI) seroconversion was observed in 42–43% of infants in the vaccine groups compared to 8% in the placebo group. Virus shedding was identified in 57–73% of vaccine recipients versus 3% of placebo recipients. The proportion of seroconversion was 78–80% in the vaccine groups and 8% in the placebo. These results show that this live intranasal bovine PIV type 3 vaccine is infectious and immunogenic. Further observations on protective efficacy are awaited.

The live-attenuated human PIV3 candidate vaccine, cp45, has been found to be safe immunogenically and phenotypically stable in infants. The genetic basis of the temperature sensitive, cold adapted and attenuation phenotypes has been defined, and genetic stability has been shown (Skiadopoulos et al., 1999). A cDNA-based recombinant cp45 containing the mutations of cp45 was constructed, and it was shown that each of the three mutations in the L polymerase protein conferred the temperature sensitive and attenuation phenotypes (Tao et al., 1998). A recombinant which possessed each of the cp45 mutations except those in L was temperature sensitive and attenuated in hamsters. These findings indicate that multiple mutations within the genome contribute to these phenotypes. The composite phenotype from multiple genetic alterations contributes to attenuation and stability.

The existence of wild type and attenuated PIV3 viruses has made it possible to generate a live attenuated virus for PIV1. The coding sequences for the hemagglutinin-neuraminidase and fusion proteins of PIV3 were replaced with those of PIV1 in the PIV3 genomic background. This rPIV3-1 virus bears the major protective antigens of PIV1. A derivative carrying the temperature sensitive change in the *L* gene of the cp45 PIV3 live attenuated virus has also been constructed (Whitehead et al., 1998). Infection of hamsters with this virus, called rPIV3-1 cp45L, generates a moderate level of antibodies against PIV1 but complete resistance to challenge with wild type PIV1. Thus a reverse genetic system has been used successfully to rapidly create a live-attenuated vaccine candidate.

7. Antiviral drugs and treatment

7.1. Neuraminidase inhibitors

7.1.1. Zanamivir and GS4071: clinical experience

Neuraminidase is a surface glycoprotein that possesses enzymatic activity essential for the replication of influenza A and B viruses (Hayden and Gwaltney, 1994; Hayden et al., 1994). Neuraminidase is a tetrameter, and the active enzyme site is located within a pocket on the surface of each glycoprotein subunit. The active site is highly conserved across influenza A and B viruses. This enzyme is responsible for the cleavage of bonds existing between neuraminic acid and adjacent sugar residues. The main actions of this enzyme are to promote virion release from infected cells and to prevent virus aggregation. Its action also prevents inactivation of influenza virus by respiratory mucus and facilitates spread within the res-

piratory tract. At least one type of influenza neuraminidase can also bind plasminogen, which contributes to HA cleavage activation of certain strains. Based on the crystallographic structure of NA, two neuraminidase inhibitors have reached advanced clinical development: inhaled zanamivir (GG167) and oral GS4104. GS4104 is the ethyl ester prodrug of GS4071, which is the active compound (Calfee and Hayden, 1998).

Zanamivir has oral bioavailability of less than 5% and is poorly distributed to the respiratory tract after intravenous administration in animals, in part because of rapid renal elimination. When administered by inhalation in humans, approximately 7–15% of the drug reaches the lower airways and 80–90% deposits in pharynx. It is estimated than the absolute bioavailability of inhaled zanamivir is about 10–20% (Efthymiopoulos et al., 1994; Calfee and Hayden, 1998). Inhaled zanamivir is well tolerated without known significant side-effects. Experimental infection studies in animals and humans have shown the protective effect of topical zanamivir. In experimental human influenza zanamivir given intranasally was 82% effective in preventing infection and 95% effective in preventing febrile illness (Hayden et al., 1996). Early treatment was shown to decrease virus titers in nasal wash and to decrease symptoms. Intravenous administration in experimental human infection was also highly protective (Calfee et al., 1998). The protective efficacy of inhaled zanamivir was tested in healthy ambulatory adults during an influenza outbreak. Once influenza virus was circulating in the community, inhalation of zanamivir once daily or placebo for 4 weeks, was initiated (Monto et al., 1998). The efficacy of the antiviral in the prevention of laboratory confirmed clinical influenza illness was 67%, and efficacy in prevention of influenza infection with or without symptoms was 31%. The potential role of seasonal prophylaxis is likely restricted to high-risk patients when the vaccine is likely to be ineffective due to antigenic change or poor host immune response. Post-exposure prophylaxis in facilities, and use of inhaled zanamivir for preventing nosocomial influenza in nursing home are under study.

Zanamivir has also demonstrated therapeutic activity in treating acute, uncomplicated influenza in adults. In 262 healthy adults with natural influenza, zanamivir given by intranasal spray plus inhalation or inhalation alone was compared to placebo (Hayden et al., 1997a). Zanamivir treatment provided a significant decrease in median time to alleviation of key influenza symptoms of 1 day (4 vs. 5 days in the placebo group). In the subgroups of patients with febrile illness or with symptoms of less than 30 h, there was a 3-day reduction in the time to alleviation of all major symptoms. Another recent trial confirmed these observations and found clinical benefit with a reduced risk of complication in high-risk patients with underlying pulmonary disease (The MIST Study Group, 1998). A controlled clinical trial of nebulized zanamivir in hospitalized patients with influenza lower respiratory tract disease is underway.

GS4104 has also shown clinical activity in preventing and treating acute influenza. The active compound GS4071 is poorly absorbed, but the bioavailability of the parent prodrug GS4104 is good (more than 70%) following oral ingestion. Oral bioavailability is not reduced by food, and the plasma half-life is 7–9 h so that infrequent dosing is feasible. GS4071 is generally well tolerated but is associated with upper gastrointestinal upset in about 15% of adults with acute influenza. This can be largely prevent by administration with food.

GS4071 provides protection against experimental influenza infection in animal and human studies (Hayden et al., 1997b). GS4071 has also recently showed to be protective against naturally occurring influenza when administered for 6 weeks in a healthy, working adult population (Aoki et al., 1998; Hayden et al., 1998). Once daily administration (75 mg/day) was associated with a protective efficacy of 82% against influenza illness. Among young healthy adults suffering from natural influenza, oral GS4104 treatment shortened the median duration of symptoms which was 4.3 days in the placebo groups and 2.9 in the groups receiving one or two doses of GS4104 (Treanor et al., 1998). This was associated with a 40% reduction in symptom severity.

Importantly, GS4104 treatment was associated with a reduction in physician-diagnosed complications requiring antibiotics. Further studies of oral GS4104 for treating influenza in children and in elderly and high-risk adults are in progress.

7.1.2. Resistance to neuraminidase inhibitors

Passage of influenza viruses in cell culture in the presence of zanamivir eventually leads to emergence of resistant mutants. However, selection of resistant variants requires considerably more effort than for amantadine and rimantadine. Genotypic analysis of these mutants indicates that mutations in either of the major surface glycoproteins, the hemagglutinin (HA) or neuraminidase (NA), can confer resistance to this class of compounds. The most common mutation observed on neuraminidase is a Gly substitution for a Glu at residue 119. This residue interacts directly with the guanidino moiety of zanamivir. Other substitutions at residue 119 and at catalytic site Arg292 have been found in vitro. The neuraminidase bearing mutations at 119 have reduced sensitivity to zanamivir (more than 100-fold) in neuraminidase inhibition assays. However, these variants have either reduced enzyme activity or stability. The in vitro selected zanamivir-resistant mutants possessing mutations in their neuraminidase also demonstrated reduced infectivity when tested in mice. Some mutations identified on the HA reside in close proximity to the receptor binding site, and result in the reduction of the virus affinity for cellular receptors. Such variants have decreased dependence on neuraminidase activity for release from infected cells. Because this mechanism of resistance is independent of the neuraminidase activity, the HA mutants have demonstrated cross-resistance to the other neuraminidase inhibitors in cell culture. One HA mutant resistant in vitro was fully sensitive to zanamivir in mice, and the impact of the HA mutations on the pathogenicity of zanamivir-resistant influenza viruses in humans remains to be determined.

Recently, influenza B virus was repeatedly isolated from an immunocompromised child with pneumonia despite exposure to nebulized zanamivir for 2 weeks (Gubareva et al., 1998). An HA mutation (198 Thr → Ile) was detected after 7 days of treatment. This mutation abolished a glycosylation site in close proximity to the HA receptor binding site and reduced the virus affinity for Siaα2-6Gal-receptors. The HA mutation was followed by a mutation in the neuraminidase active site of the virus (152 Arg → Lys). Arg 152 is a functional residue which directly interacts with the sialic acid moiety and participates in the substrate binding. This mutation led to a 1000-fold reduction in the enzyme sensitivity to zanamivir. The mutant enzyme activity had 3–5% of the enzyme activity in vitro compared to the wild type virus. As man and ferret share similar specificity of the receptors present on the respiratory tract epithelium (Siaα2-6Gal) challenge of ferrets was done. This experiment has shown a growth preference of the parental virus over the mutant in the untreated, although not in the zanamivir-treated ferrets. In addition, the mutant virus demonstrated a reduced virulence: a greater quantity of the mutant virus was required to establish a productive infection of ferrets when compared with the parent and the nasal wash virus titers, cells counts and protein levels were lower in the animals infected with the mutant virus.

Some virus variants behaved like the wild type virus, suggesting that different receptors are used in MDCK cells and in vivo and that some HA mutations have no impact. For example, the influenza B virus from the immunocompromised child was sensitive in a standard assay in MDCK cells. The mutant virus has an increased affinity for MDCK cell receptors which in combination with a reduced neuraminidase activity renders this virus sensitive to zanamivir in this cell culture. These data indicate that the current methods for monitoring resistant mutants are potentially flawed due to the absence of a cell culture system that adequately reflects the human respiratory tract epithelium. The neuraminidase inhibition assay detects emergence of zanamivir-resistant mutant viruses possessing the resistant enzyme, but a practical methods for monitoring HA and NA mutations that convey resistance is needed.

7.2. Influenza prophylaxis in the nursing home

Influenza is a leading cause of death in the elderly ranking to between the third and fifth cause of death in adults of 65–85 years of age. Clinical presentation in this population is not specific and fever occurs in only 60–75% of cases of influenza in elderly patients. Annual influenza vaccination decreases the rates of lower respiratory tract disease, hospitalization, and death in nursing homes, but influenza vaccine has been estimated to be only 30% effective in frail nursing home residents (Patriarca et al., 1987). Prospective surveillance has documented annual outbreaks of influenza despite resident vaccination rates of $>85\%$. Recommendations from the Advisory Committee on Immunization Practices for an influenza outbreak control program include antiviral chemoprophylaxis to limit influenza outbreaks in nursing homes (Advisory Committee on Immunization Practices, 1997).

The effectiveness of short duration prophylaxis for nursing home influenza outbreaks was tested in a 4-year study in a rural, 700-bed home for veterans. Volunteers were randomized to short duration (minimum of 14 or 7 days after the last case in the facility) or long duration (minimum of 21 or 7 days after the last case in the facility) amantadine or rimantadine during outbreaks of influenza A (Drinka et al., 1998). Criteria for an influenza A outbreak were met in 3 of 4 years. A total of 32 culture-confirmed cases were identified during 28 days in 1991–1992, 68 cases were identified during 51 days in 1993–1994, and 12 cases were identified during 33 days in 1994–1995. No participants developed influenza after the termination of short- or long-term prophylaxis despite persisting influenza in the community. Antiviral chemoprophylaxis can be effective in controlling nursing home influenza outbreaks when administered for a period of 7 days beyond the last culture-confirmed influenza case in a nursing home. However, amantadine-resistant strains were identified in 13 residents; seven developed illness while on prophylaxis, and six had not received antiviral medication. In addition, a retrospective chart review found two- to fourfold increase in falls, agitation, and confusion during amantadine administration. A placebo controlled trial showed rimantadine to be associated with an increase in the number of significant health events including death in a study of long-term use of rimantadine for seasonal prophylaxis in nursing homes (Monto et al., 1995). Such experiences highlight the need for alternative antiviral agents.

A prospective, randomized unblinded pilot study was performed to examine the safety and efficacy of zanamivir in influenza outbreak control in a nursing home (Schilling et al., 1997). A 14-day chemoprophylaxis with zanamivir was compared to standard of care during sequential influenza A and influenza B outbreaks in a 735 bed nursing home. A total of 65 volunteers on four epidemic units were randomized to zanamivir and 23 volunteers were randomized to rimantadine on two epidemic units. During the 14 days of prophylaxis, only four new febrile respiratory illnesses were detected. One volunteer receiving rimantadine developed influenza. In another study 35 volunteers on two units with influenza B outbreaks were randomized to receive zanamivir and 18 volunteers on one epidemic unit were randomized to no drug. During the 14 days of prophylaxis, only one new febrile respiratory illness was detected. No serious adverse events occurred. A large multicenter, double-blind, randomized, controlled trial is underway to investigate the efficacy and safety of inhaled zanamivir in controlling nursing home influenza outbreaks.

7.3. Ribavirin

Despite the synthesis of ribavirin nearly 30 years ago, precise mechanisms of its action remain incompletely understood. Ribavirin exerts its antiviral effect via inhibition of inosine monophosphate dehydrogenase resulting in reduction in the guanosine triphosphate (GTP) pools necessary for transcription of viruses, but the drug also appears to interfere with 5′ capping of mRNA, thereby inhibiting GTP-dependent mRNA transcription. Furthermore, ribavirin has been shown to directly inhibit the initiation and elongation of virus-specific RNA polymerase. Emergence of resistance does not occur. Ribavirin administration was adapted for inhalation by the development of the

small particle (<4 μm) aerosol generator (SPAG). Small, water aerosol-controlled studies in infants hospitalized with RSV bronchiolitis and pneumonia led to licensure of the drug for RSV disease in children. The use of ribavirin in young infants has become increasingly controversial due to the lack of large clinical randomized trials with appropriate controls showing effect on clinical relevant endpoints. Concerns regarding bronchospasm, exposure of health workers and high costs also have contributed to its decreasing use. Aerosolized ribavirin is becoming decreasingly utilized in otherwise healthy pediatric patients but its use in immunocompromised patients continues.

Unfortunately controlled studies of the treatment of RSV in high-risk immunocompromised patients has not been undertaken; when compared with historical controls use of ribavirin monotherapy in adult bone marrow transplant does not indicate benefit. The addition of IVIG to aerosolized ribavirin is associated with 30% mortality in RSV pneumonia, which is less than historical controls. Intravenous ribavirin does not seem to be associated with benefit in RSV pneumonia. Ribavirin given early before any radiologic signs seems to be associated with some benefit in treating RSV infection in immunocompromised hosts. The potential of preemptive therapy to prevent-treat RSV disease in BMT patients is being investigated in a prospective, blinded but not placebo-controlled trial under the auspices of The Collaborative Antiviral Study Group (CASG). The benefit of ribavirin probably does exist only if the treatment is given early with high dosages.

Ribavirin is not licensed for the treatment of influenza and although demonstrated to be effective in college students, one trial found little benefit in children hospitalized with influenza. Ribavirin has been demonstrated to be effective when given intravenously for systemic hemorrhagic fevers, such as Lassa fever, and Hantaan hemorragic fever with renal syndrome. In these instances, large, well controlled clinical trials were conducted utilizing mortality as a clinical endpoint. Case reports of efficacy have been described for Crimean-Congo hemorrhagic fever virus and the Sin Nombre Virus or Hantavirus pulmonary syndrome. Ribavirin is reasonably active in vitro against measles and adenoviruses; but clinical reports, mainly in immunocompromised hosts are uncontrolled. Ribavirin was also tested in BMT patients with PIV infection without showing an obvious clinical benefit.

7.4. New antiviral agents

7.4.1. Protease inhibitors and anti-picornavirus agents

The Picornaviridiae family consists of more than 200 serotypes, which are associated with the common cold, aseptic meningitis, conjunctivitis, encephalitis, and respiratory disease. Human rhinoviruses include 100 different serotypes. No effective therapies are currently available but several new compounds are under development.

The rhinovirus 3C protease is responsible for the cleavage of viral precursor polyproteins into structural and enzymatic proteins. DNA sequence comparisons among rhinovirus serotypes, and even among several related picornaviruses, have identified a significant degree of homology within the 3C coding region including strict conservation of the active site residues. Resolution of the three-dimensional structure by X-ray crystallography has demonstrated that 3C protease has the polypeptide backbone of a serine protease but with the active-site nucleophile comprised of a cysteine sulfhydryl. AG7088 is an irreversible, peptidomimetic inhibitor of 3C protease.

In cell protection assays, AG7088 inhibited the replication of 48 rhinovirus serotypes with a mean EC_{50} of 23 nM and inhibited also replication of related picornaviruses including coxsackieviruses A21 and B3, enterovirus 70 and echovirus 11. The 50% cytotoxic dose was >1000 μM yielding high therapeutic indices. Direct inhibition of 3C proteolytic activity showed a dose-dependent accumulation of viral precursor polyproteins and reduction of processed protein products. Treatment of a rhinovirus infected human bronchial epithelial cell line resulted in a dose-dependent reduction of both infectious viruses as well as IL-6 and IL-8 elaboration. In vitro the efficacy of AG7088 compares favorably to other drugs in development such as pleconaril. The AG7088

anti-protease is poorly absorbed and a nasal formulation has been developed. Clinical studies are expected in the near future.

Pleconaril is an oral compound, which is active against picornaviruses, namely rhinoviruses, coxsackieviruses and echoviruses. The mechanism of action of this agent is to bind to the capsid of the virus inhibiting attachment or virus uncoating. The in vitro sensitivity of rhinoviruses, coxsackie and echoviruses is generally <1.0 mg/ml. Pleconaril is well absorbed with bioavailability of 70%. After a single oral dose of 200 or 400 mg the plasma concentration is >0.5 mg/ml. The pharmacokinetic profile is similar in adults and in children. Pleconaril is lipophilic, and in animal studies, the concentrations in the meninges and nasal epithelium are greater than in plasma. Experimental infection studies with coxsackie A21 virus have been done in 33 patients randomized to receive 400 mg of pleconaril or placebo beginning before virus exposure. In patients receiving oral pleconaril few symptoms were observed compared to placebo, although subjects did become infected. Phase 2 studies have been completed in 39 patients with viral meningitis (PCR for any virus was positive in 85% of cases). The duration of the disease was shorter in the treatment group compared to the placebo group, 5 days versus 10.8 days. Among pediatric patients with enterovirus meningitis, a significant decrease of the global score of morbidity and a better clinical recovery were found in placebo-controlled trials. Open label compassionate studies suggest that pleconaril provides a clinical benefit among agammaglobulinemic patients with chronic enterovirus encephalitis. Controlled clinical trials evaluating oral pleconaril in adults, including asthmatic patients, and children with suspected rhinovirus diseases are in progress.

Intracellular adhesion molecules (ICAM) have shown to be a major receptor for rhinoviruses and soluble ICAM-1 a specific inhibitor of rhinovirus replication. In monkeys soluble ICAM-1 prevents infection with rhinoviruses. In 177 humans infected with rhinovirus 39 intranasally soluble ICAM-1 was shown to decrease virus titers and to prevent cold symptoms. However these effects were not significant, and development was stopped.

7.4.2. Paramyxovirus fusion protein inhibitors

Virus-specific fusion proteins mediate virion membrane fusion to host cell and from infected cell to uninfected cell. These proteins are required for virus infection and spread within the host (White, 1992). Synthetic peptides derived from predicted regions within the trans-membrane protein of HIV-1 are potent inhibitors of viral fusion and infection in vitro (Wild et al., 1993). One of those peptides, T20, derived from a gp41 domain, has already demonstrated inhibition of HIV-1 replication in human trials.

Based on the amino acid motif of active peptides from HIV-1 transmenbrane protein, similar protein sequences were sought in other fusogenic proteins. The procedure used a 'computerized antifusion searching technology' allowing identification of similar domains in all viral sequences in the Swiss-Prot database. This strategy accurately predicts sequences of the surface glycoproteins of fusogenic viruses including HIV, influenza, hepatitis B and C and the paramyxoviruses (Lambert et al., 1996).

Paramyxoviruses contain two major surface glycoproteins: a receptor binding protein (HN for parainfluenza, H for measles or G for RSV) which facilitates attachment to cells and a fusion protein, required for release of the genome-containing nucleocapsids into the cell host cytoplasm (Joshi et al., 1998).

The research identified active peptides within both heptad repeat 1 and 2 domains of the fusion 1 protein from PIV3 and RSV. These domains include predicted helical sequences identified in some retroviruses and shared several properties with the HIV-1 gp41 domains. Activity of these proteins seems to be specific without cross reactivity (Yao and Compans, 1996).

Active peptides had antiviral EC_{50} values for these paramyxoviruses ranging from 0.05 to 0.5 μM for infectivity and fusion inhibition in vitro. No associated cellular cytoxicity was identified. The RSV peptide from the heptad repeat 2 domain has in vivo efficacy in the cotton rat model of RSV infection. The accumulating literature of active peptides from fusogenic proteins strengthens the concept that functionally similar domains exist in retrovirus, orthomyxovirus and paramyxovirus fusion proteins.

8. Summary

Viral respiratory tract infections are a leading cause of morbidity and mortality worldwide. In many instances RT-PCR has shown that the frequency of respiratory tract infections associated with particular respiratory viruses is higher than suspected. These techniques have established that common respiratory viruses are the principal cause of acute otitis media, sinusitis, and asthmatic exacerbation. The associated excess use of antibiotics for these presumed bacterial infections has contributed to health care costs and fostered emergence of resistant bacteria. Certain populations, particularly the elderly, transplant and leukemia patients, and those with asthma and underlying lung diseases, are at particular risk for more severe disease due to common respiratory viruses.

The outbreak of H5N1 influenza viruses in Hong Kong in 1997 reminds us that the threat of a new deadly influenza pandemic is ever present. Molecular studies showed that this influenza virus was an avian strain and was transmitted to humans without intermediate hosts. The containment of this threatening outbreak was achieved by the destruction of the domestic poultry reservoir of this H5N1 virus. This outbreak emphasizes the need to support effective public health programs and to continue influenza surveillance.

The clinical syndromes and severity of illness manifested after respiratory viral infection reflect differences both in virulence among different respiratory viruses and in host responses. Observations on the pathogenesis of RSV infection have shown that the host immune response contributes to clinical manifestations and to the development of bronchiolitis, and clinical trials suggest that antivirals may not be sufficient to control the disease process. The complexity and redundancy of the cytokine network indicate that more information is needed about the dynamic and pattern of host inflammatory responses in diverse syndromes like common colds, influenza, and bronchiolitis.

Several rapid diagnostic tests for influenza A and B viruses, based on detection of nucleoproteins or neuraminidase activity, are in various stages of development or clinical use. While the specificity of these tests is generally good, their sensitivity is variable and depends on the type of samples and the age of the patient. Rapid assays are useful for surveillance, but conventional cultures are necessary to identify circulating influenza strains.

A new class of antiviral drugs effective against influenza A and B viruses are in advanced clinical development. Neuraminidase inhibitors, namely inhaled zanamivir and oral GS4071, have proven to be effective drugs to reduce symptoms and potentially to reduce the risk of complications of acute influenza infection. Prevention of influenza in healthy adults is effective if the prophylaxis is used during the epidemic period. These agents could provide an useful adjunct to vaccines for prevention. Results of ongoing studies in elderly, immunocompromised hosts, and children in whom influenza is associated with significant morbidity or mortality, are awaited with interest.

Influenza virus strains resistant to neuraminidase inhibitors have been selected in vitro. Resistance results from mutations in the HA and/or neuraminidase genes and occurs after prolonged exposure to the drug in vitro. Observations in cell culture and in experimentally infected animals indicate that neuraminidase variants replicate less well than wild type strains. Resistant variants have been novely detected during clinical use and the significance of neuraminidase resistant strains needs further evaluation.

Effective antiviral agents are needed for a number of other respiratory viral pathogens. Although aerosol ribavirin has been used in hospitalized children, its efficacy in treating RSV bronchiolitis is controversial and better agents are needed. No agent of proven value is currently available for rhinovirus, coronavirus, PIV, or adenovirus infections. Pleconaril is an anti-picornavirus, capsid-binding agent, which has shown promising preliminary results in treating enterovirus meningitis. Clinical studies are ongoing in presumed rhinovirus infections involving asthmatic and otherwise healthy persons. Inhibitors of the 3C protease of rhinovirus have been identified and clinical studies using topically applied antiprotease agents are expected in the near future.

The live-attenuated, cold-adapted influenza vaccine represents a major clinical advance. A trivalent formulation of this influenza vaccine has been shown to be highly protective against influenza illness and its associated complications in children. During the 1997–1998 season it was also found to protect against a drift variant in both children and adults. The cost-effectiveness of annual vaccination of all healthy adults has not been clearly established. Global health strategies should probably continue to focus mainly on improving vaccination in high-risk populations.

The use of molecular biology has allowed the construction of recombinant cold-adapted attenuated RSV viruses, which elicit good immune responses. Clinical studies are ongoing in young infants and children. Phase I and II studies using a live intranasal bovine PIV type 3 vaccine have also been conducted in children and in adults and further clinical trials are ongoing. In contrast, initial results with adjuvants and DNA vaccines for influenza have indicated the need for further investigations to develop more immunogenic formulations.

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