1.0
Introduction
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1.0 Introduction

1.1 Viruses

The virus is an elementary biosystem that possesses some of the properties of living systems such as having a genome and being able to adapt to changing environments. However, viruses cannot capture and share free energy and they are not functionally active outside their host cell. They are distinguished from other microbes due to their simple organization, characteristic mode of replication and obligate parasitism.

A mature and potentially infectious particle is called virion. Virions are made up of two essential constituents: a genome and a capsid. The genome is made up of nucleic acid, it may be single stranded or double stranded, DNA or RNA and may exists as one or more molecules. The single stranded molecule may have positive or negative polarity. The genome is associated with protein capsid. The genome with capsid is called nucleocapsid. Nucleocapsid is sometimes surrounded by a lipid bilayer or an envelope with glycoprotein embedded in it. The virions exhibit different morphological symmetries viz. icosahedral, helical, enveloped and complex type. They exhibit host specificity during infection and produce characteristic effect on host cells i.e. death, fusion or transformation to cancer cells.

1.1.1 Classification

Classification of viruses is based on the properties like virion morphology, genome organisation, mode of replication, the number and size of structural and non-structural viral proteins. These are family or genus defining properties.

Viruses with apparently common evolutionary origin, and common characteristics are placed in common group called genus (Suffix-virus i.e. Pneumonivirus). A group of genera with common characteristics represents a family (suffix-viridae eq. Paramyxoviridae). For discrimination of species of the same genera following characters are useful viz, genome sequence relatedness, natural host range, cell and tissue tropism, pathogenecity and cytopathology, mode of transmission, physicochemical properties of virion, antigenic properties of viral proteins.

International Committee on Taxonomy of viruses (ICTV, 2000) has approved 3 orders, 56 families, 9 subfamilies, 233 genera and 1550 virus species. More than 60 genera and about 25 families of human and animal viruses are now recognized.
1.2 **Respiratory Viruses**

Acute respiratory infection (ARI) is the leading cause of morbidity and mortality throughout the world particularly in developing countries (Selwyn, 1990). Majority of the severe infections are due to lower respiratory tract infection (LRTI). It is estimated that ARI causes annually over 4 million deaths and millions of episodes of illness in children less than 5 years of age in developing countries (Pio, 1988). The incidence of total respiratory infections is similar in developed and developing countries whereas mortality is significantly more in developing countries (Dolin and Wright, 1999). Agents causing acute respiratory illnesses are same all over the world wherever investigation have been conducted and viral pathogen account for 30-40% of ARI cases (John et al., 1991; Huq et al., 1990).

Viral ARI occurs worldwide and the viruses that primarily infect the respiratory tract are called as ‘Respiratory viruses’. **Figure 1** indicates infections of human upper and lower respiratory tracts.

**Figure 1: Viral Infections of the Respiratory Tract**

- Common cold / Rhinitis
- Pharyngitis
- Laryngitis / croup
- Tracheobronchitis
- Bronchiolitis
- Pneumonia

Respiratory viruses have three patterns of replication in the respiratory tract, viz.

- Acute infection with growth confined to the respiratory mucosal surface.
- Persistent replication or latency on the respiratory mucosal surface.
- Primary replication on the respiratory epithelium as a preamble to systemic spread.
There are over 200 human respiratory viruses, falling mainly within six families *Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Coronaviridae, Adenoviridae* and *Herpesviridae*. The viruses most frequently involved are respiratory syncytial virus (HRSV), influenza viruses (Flu), parainfluenza viruses (PIV), adenoviruses, coronaviruses (CoV) and rhinoviruses, which causes upper as well as lower respiratory tract infection with severe ALRI in children. In adult viral pneumonia is not very common but immunocompetent and immunocompromised adults do develop viral pneumonia (Ruben, 1993) however, at the extremes of age the impact of these viruses can be most devastating (Dhar *et al*., 1976). Table 1 shows, relative importance of viruses of upper and lower respiratory tract disease.

**Table 1: Involvement of different respiratory viruses in respiratory tract diseases**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Common cold</th>
<th>Flu/Flulike illness</th>
<th>Otitis media</th>
<th>Bronchiolitis</th>
<th>Pneumonia</th>
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<tr>
<td>Influenza</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Parainfluenza</td>
<td>+++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>HRSV</td>
<td>+++</td>
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<td>+++</td>
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<tr>
<td>Adenovirus</td>
<td>+++</td>
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<td>-</td>
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<tr>
<td>Rhinovirus</td>
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<td>+</td>
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<tr>
<td>Coronavirus</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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*Adapted from 'Clinical Virology Manual' 3rd edition, 2000*

+ to +++ minimal or major importance, - no or negligible importance

As a group, these viruses annually account for substantial morbidity and mortality. Persons of all age groups are susceptible to respiratory tract infection although children suffer about twice than adult. These infections also represent an important cause of days lost from work or school, accounting significant losses both socially and financially. Many factors contribute to the severity of illness including characteristic of virus, inoculum size, and host factors such as age, general health, underlying disease, socioeconomic, nutritional, and immune status.

The etiologic diagnosis of viral ARI requires laboratory confirmation. Along with the old techniques like tissue culture isolation and serological tests like complement fixation test (CF), several new diagnostic tests for respiratory viruses are commercially available or are under development. These tests include polymerase chain reaction
(PCR), monoclonal antibody (MAb) based enzyme immunoassays (EIA),
immunofluorescence tests (IF) and an optical immunoassay for specific and rapid
identification of individual virus infection (Kellogg, 1991; Chomel et al., 1992;
Dominquez et al., 1993; Olsen et al., 1993b; Leonardi et al., 1994; Covalciuc et al.,
1999).

Respiratory virus infections represent a major public health problem because
of their mode of transmission (usually by aerosol, direct contact, or by fomites),
worldwide occurrence, considerable morbidity and mortality with rapid spread in
communities. This has stressed the need of effective and safe therapeutic drugs and of
highly immunogenic and protective vaccines for each of the individual respiratory
viruses.

1.2.1 Influenza viruses

Influenza viruses are Orthomyxoviruses. They are classified into three
distinct types, Influenza A, B and C. There are significant differences in genetic
organization, antigenic properties, host range, epidemiology and clinical characteristics
among the three virus types. However, all three viruses share certain characteristics,
which are fundamental to their biological behavior including the presence of a host cell
derived envelope.

Influenza is an acute respiratory tract infection that usually occurs in
epidemics. Antigenic changes continuously take place within the group of influenza A
than group B. Influenza C appears to be antigenically stable. Influenza A virus undergoes
two distinct forms of antigenic variation. Antigenic drift reflects minor antigenic changes
in either the haemagglutinin (HA) or neuraminidase (NA) or both. Antigenic shift, is
major change occur infrequently and reflects the appearance of viral strains with surface
antigens that are immunologically only distantly related to those of earlier strains. The
antigenic shift may involve either haemagglutinin alone or the neuraminidase as well.

Clinical Features: Infection spreads from one person to another by the
aerosol route by small particle droplets or by fomites. In the course of uncomplicated
disease, symptoms like fever, chills, generalized aching (muscular), headache,
prostration and anorexia are more prominent. Influenza is self-limited disease lasting 3 to
7 days; about 10% of patients with clinical influenza have lobular pulmonary
consolidation. Secondary bacterial pneumonia is major cause of deaths. Combined
influenza virus-bacterial pneumonia is approximately three times more common than primary influenza pneumonia.

**Disease Impact:** Both influenza A and B are associated with severe illness. Attack rates are generally highest in young where as mortality is generally highest in elderly. Excess morbidity and mortality are particularly high in those with certain high-risk conditions such as a person with cardiovascular and pulmonary condition including asthma, chronic metabolic disease, renal dysfunction, haemoglobinopathies or immunodeficiency (Safrin et al., 1990).

**Epidemics and Pandemics:** Epidemics and pandemics of influenza are known to spread through travel routes. The incidence of influenza has been recorded in many countries during past 400 years. Many of these epidemics were characterized by their rapid spread and explosive nature. It is known that no other epidemic of febrile respiratory diseases mimics influenza epidemics.

Pandemics are caused by shift of human influenza type A virus and occur at 10-40 years interval. Frequent drifts of pandemic strain occur and cause epidemics. Influenza epidemic occur in waves. The inter-epidemic period for type A appears to be 2-3 years and type B, 3-6 years. They usually begin abruptly; reach a sharp peak in 2 to 3 weeks and last 6 to 10 weeks. During epidemics, the overall attack rate is about 10-20%. School children are mainly affected. Attack rate of 40 to 50% are seen in closed communities like nursing homes, hospitals etc. Frequent epidemics occur after pandemics, as a result of minor antigenic variation of the pandemic virus strain. Minor antigenic variant strains of type A (H1N1), A (H3N2) and type B influenza virus are currently circulating globally, causing frequent epidemics.

History of different pandemics includes. Swine influenza 1918, Asian influenza pandemics 1957, Hong Kong influenza pandemic 1968 and Russian influenza pandemic 1977. Swine influenza pandemic was a killer pandemic with fatality rate of 3%. According to rough estimate about 20 to 50 million people, mainly young adults, died during this pandemic alone. A recent study employing PCR suggest that it was an H1N1 influenza A virus. Asian influenza pandemic, 1957 was caused by a strain H2N2. It was mild as compare to Swine influenza pandemic of 1918. Hong Kong influenza pandemic 1968 was caused by a new strain of H3N2. Russian influenza pandemic 1977 was re-emergence of H1N1 in USSR and subsequently outbreaks caused by this virus were reported from all over the world. During this pandemic simultaneous circulation of two subtypes H3N2 and H1N1 of influenza A was observed.
Bird influenza outbreaks (H5N1 and H9N2 outbreak in Hong Kong): Transmission of avian influenza viruses to humans in recent years has been observed in Hong Kong in 1997. Thousands of chickens died in Hong Kong after infection with H5N1. The outbreak was brought under control by mass slaughtering of approximately 1.6 million chickens and other birds in Hong Kong during December 1997.

Considering the public health importance of influenza, in 1947, World Health Organization (WHO) initiated a network of surveillance centers all over the world. In India, national influenza centers are located at National Institute of Virology (Pune, Maharashtra), Central Research Institute (Kasauli, Himachal Pradesh), and Pasteur Institute (Coonoor, South India). These institutes have investigated and reported pandemics and epidemics caused by global influenza virus strains during the past 50 years (Rao, 2003).

**Diagnosis:** Presumptive diagnosis can be made from clinical and epidemiologic considerations. Isolation of virus in cell cultures from samples of respiratory secretions such as nasopharyngeal swabs, washes or throat swabs remains gold standard for viral diagnosis. Renal epithelial cells and Madin-Darby canine kidney (MDCK) cells are generally used. Virus can be isolated in embryonated hen’s eggs that require 3-5 days.

A variety of other techniques include IF and enzyme linked immunosorbent assays (ELISA) for the detection of antigen from clinical specimens. Commercially available reagents include filter immunoassay (Directigen FLU-A) and microtiter plates assays (Enzyanost influenza A and B, Germany). Recently PCR has been described for rapid detection of virus from clinical specimen and also for characterization of viral genes without alterations introduced by virus isolation tests.

**Vaccine:** Inactivated influenza vaccine: The vaccine comprises the current strains of type A and B viruses grown in chick embryos and inactivated with formalin or β-propiolactone, for parenteral use in humans. Inactivated viral vaccines usually do not generate good local immunoglobulin A (IgA) or cell-mediated immune responses. Following immunization, protection is about 70% or less for a year. Immunity against the homologous virus strain appears to be of short duration, probably 1-3 years. These vaccines have to be regularly updated because of antigenic variation. Annual vaccination is recommended for persons belonging to the high-risk category.

**Live attenuated vaccines:** The use of live attenuated viruses as influenza vaccines offers several potential advantages, including induction of mucosal immune
response, which closely mimics that induced by natural influenza viral infection. It also gives protection of upper respiratory tract (URI).

**DNA vaccine:** Recently immunization of African green monkeys with DNA encoding combination of three HA from influenza virus genes has shown to induce serum antibodies against all HA (Donnelly *et al.*, 1995). Studies of similar candidate vaccine have been initiated in humans.

**Antiviral therapy:** Amantadine (1-adamananamine hydrochloride) and the related drug rimantadine (α-methyl-1-adamanine methyl amine hydrochloride) are currently licensed for the prevention and treatment of influenza. A compound 4-guanidino 2, 4-dideoxy, 2, 3-dehydro-N-actyl neuramine acid (GG 167) is highly potent and selective inhibitor of the neuraminidase of influenza A and B. GG 167 has significant antiviral activity against a wide variety of laboratory and clinical influenza A and B isolates. Limited studies in humans have also indicated the efficacy of GG167 (Hayden *et al.*, 1996).

### 1.2.2 Parainfluenza viruses (PIV)

Parainfluenza viruses (PIV) were discovered in late 1950s (Chanock *et al.*, 1958). It was subsequently distinguished into four separate serotypes (1,2,3 and 4), which cause respiratory disease. Types 1, 2 and 3 are clinically most important serotypes and type 4 has been implicated only in upper respiratory tract disease.

PIV has been classified under the family Paramyxoviridae. As a group, PIV comes second only to HRSV. PIV has a strong impact on medically immunosuppressed adult population where it causes progressive pneumonitis. In healthy adults or in children, infection with PIV-1 or PIV-3 is mild to moderate upper respiratory infection with symptoms of nasal discharge, obstruction, sneezing and erythema of nasal passage or throat. In children it is often accompanied by otitis media. Among the group, PIV-3 has a broader tropism and can cause bronchitis and pneumonia in young infants, in this respect PIV-3 is most like HRSV.

PIV share a number of structural, replicative, pathogenic and clinical properties with influenza and HRSV, like reinfection throughout life. Children in the first year of life with primary infection caused by parainfluenza type 1,2 or 3 may have illness ranging from laryngotrachitis, bronchitis and croup. Virtually all infants have maternal antibodies to PIV in serum, yet they do not prevent infection or disease. Reinfection in children and adult is common which is non-febrile upper respiratory infection.
**Epidemiology:** PIV-1 and 2 causes fall epidemic however; PIV-3 is endemic in a community. In a study at Vanderbilt University Medical Center approximately 1500 children with febrile upper respiratory tract disease, otitis media, or lower respiratory tract signs, followed with viral cultures, 17% of children under age 2 had a PIV isolated, 9% PIV-3, 5% PIV-1, 2% PIV-2 and 1% other PIV strains. Of the 293 PIV isolates, 16% were associated with LRTI. PIV-1 caused croup where as PIV-2 and PIV-3 caused pneumonia and bronchiolitis (Reed *et al.*, 1996).

**Diagnosis:** PIV grows well in primary rhesus monkey or cynomolgus kidney cells or in the rhesus monkey kidney cell line (LLCMK-2) (Frank *et al.*, 1979), which can be detected by CPE, syncytium formation, haemadsorption with guinea pig RBCs, which can be confirmed by inhibition of haemadsorption or fluorescent staining of infected cells. In rapid methods, IF test using virus specific antibodies on infected cells from nasal secretions give fast results but they vary in sensitivity. Currently PCR has been used to characterize PIV isolates and may represent future of diagnostic virology (Karron *et al.*, 1994).

**Therapy:** Nebulized steroids suggest a rapid improvement in the respiratory embarrassment of mild to moderate croup, when the croup is spasmodic than infectious (Klassen *et al.*, 1994). In animal models, aprotinin, a serine protease inhibitor has decreased viral shedding and improved clinical outcome in paramyxovirus pneumonia (Ovcharenko and Zhirmov, 1994). This has an attractive future approach to therapy.

**Prevention:** Prevention of nosocomial spread of PIV infection is careful hand washing by health care personal and cohorting of hospitalized patients with documented PIV infection. For the prevention of PIV infection more recent work has focused on the attenuated vaccine both cold adapted as well as temperature sensitive mutants (Coelingh *et al.*, 1988). Vaccine candidates exhibit a comparable degree of attenuation and appear safe and immunogenic in young seronegative children and both are advancing through phase 1 and 2 trials.

1.2.3 **Adenovirus**

In 1953, Rowe first described a filterable agent, from surgically removed adenoidal tissue of children in culture. Due to their frequent isolation from adenoids the name Adenovirus was adopted. They come under to family *Adenoviridae*. The adenovirus is non-enveloped; double standard DNA virus with icosahedral capsid,
composed of hexons and pentons. The hexon protein and fibers protruding from pentons are major adenoviral antigens.

Adenoviruses are widespread in nature; contaminated water or aerosol infection may begin in eyes, nasopharynx or lungs and spread to other organs by viremia. To date, 49 different serotypes of human adenoviruses have been isolated which are divided into 6 subgenera, A-F, according to their structural, biochemical and immunological characteristics and oncogenic potential (Wandell, 1984). Among the subgenera, B and E appear in outbreaks of respiratory disease in military recruits and at times can cause fatal infections in infants. Subgenus B is also associated with outbreaks of pneumonia. (Trentin et al., 1962; Dingle and Langmair., 1968). Subgenus C is the most common cause of URTI and LRTI in small children. Some serotypes from C can infect adenoids and tonsils persistently.

Pathogenesis: In human infection, adenovirus infected cells may become damaged and die producing disease of various severity. It may cause acute infection or may lead to chronic persistent infection and latency. The latent infection may not yield whole virus but certain viral DNA expressed intermittently which might become important in maintaining latency. The same type of adenovirus rarely produces a second attack of disease. This type of persistent type specific immunity is unusual among respiratory disease.

Acute infection: Human adenovirus most commonly infects cells of the respiratory tract but the virus can be recovered from cells of other organs. Histopathological evaluation reveals extensive destruction of bronchial epithelium and bronchial glands from acute fatal adenoviral respiratory tract infection.

Chronic persistent infection and latency: Human adenoviruses can produce persistent infection in which viral shedding can be observed after the clinical symptoms subside. Tonsilar tissue is the main target where members of group C are most frequently found in such condition. Latent adenovirus may contribute to chronic airway obstruction where early protein of adenovirus found to play a major role (Matsuse et al., 1992).

Epidemiology: Human adenoviral infections occur throughout life and adenoviruses account for approximately 5-10% of febrile acute respiratory disease. Epidemic caused by respiratory adenoviruses commonly occur in institutional settings or it is associated with crowding. In crowded environment of military training camps the virus spreads via respiratory droplets. Example is, epidemic infection of military recruits by adenovirus in 1950s and 1960s where the attack reached 90% in barracks of
susceptible military recruits, accounted for 80-90% hospital admission (Hillman, 1957). Young children in crowded environment also favor adenoviral spread and develop febrile adenoviral respiratory illness within two weeks after exposure.

Immunocompromised patients or patients with kidney and lung transplant recipient or bone marrow transplant recipient are not only at risk for infection of adenovirus associated with respiratory disease but also are at the risk for opportunistic adenoviral disease.

Clinical Manifestations: Adenoviruses account for at least 7% of childhood acute respiratory infections and 17% of infant diarrhea (Brandt et al., 1969; Wandell, 1984). A significant portion of serious respiratory disease found in young children appears to be associated with the pediatric adenoviruses types 1, 2, 5, 6 and 8. Adenovirus most commonly infects the respiratory tract, causing mild and self-limited nasal congestion, coryza and cough. Virus more often produces pharyngitis and tonsillitis and is also associated with the complication of otitis media in children (Ma and Mathews, 1993). Serotypes 3, 4, 7 and 21 are most common in croup or pneumonia but most feared complication of LRT is bronchiolitis obliterans, which leads to irreversible narrowing of small airways (Becroft, 1971). However, fatal pneumonia and disseminated disease have been reported in infants, young adults and immunocompromised patients.

Diagnosis: In children younger than 3 years of age, small discrete white spots of exudates appear on tonsils, which are consistent with adenovirus infection. Diagnosis involves virus isolation, which can be confirmed by IF, CF test and detection of viral nucleic acid from clinical specimen. Type specific antibody can be used in IF or ELISA for the recognition of isolate. Characterization of viral DNA by both, hybridization and restriction endonuclease digestion pattern has also been used for clinical isolates. Currently PCR assays are being used for the diagnosis of adenovirus infection in various tissue specimen and body fluids. Electron microscopic (EM) morphology of adenovirus is unique, hence EM of clinical specimens has been used in special situations to identify viral agent rapidly and specifically.

Therapy: No antiviral treatment is available for adenoviral infection. The polyclonal immunoglobulin products are not useful in the treatment of adenovirus infection.

Vaccines: Adenoviruses were propagated in diploid strains of human embryonic lung cells (WI-38) and administered in enteric-coated gelatin capsules. The live viruses establish a silent infection in intestinal tract without any respiratory
symptoms. This vaccine has been shown to be safe, immunogenic and about 80% effective in type specific adenoviral disease in military populations (Peckinpaugh et al., 1968). The early administration of vaccine is critical. This strategy of vaccination has not been implemented in children, as there is some concern of potential oncogenesis of the adenoviruses.

1.2.4 Coronaviruses

Virus of this family is spherical or pleomorphic particle with a halo of glycoptoteins giving the appearance of corona of spikes. Coronaviruses (CoV) cause a wide range of infections in humans and other animals, including gastroenteritis, hepatitis, encephalomyelitis and tracheobronchitis (Holmes and Lei, 1996). Infection of humans is implicated in gastroenteritis, lower respiratory tract infections, rare cases of encephalitis and consistent association with upper respiratory infection. They cause 10-30% of upper respiratory infections in all ages that are more serious than rhinoviruses (McIntosh, 1996).

Two antigenically distinct prototypes of CoV are 229 E and OC43. Glycoptoteins E1, E2 and protein N, are the three major antigens of CoV.

In patients with CoV infection there is increased nasal secretion. Microvascular exudation and mucosal responsiveness are the hallmarks of the response to CoV infection and might be involved in asthma exacerbation by CoV (Greiff et al., 1994). Antibodies give some protection but not complete which wanes over the time and the patient is vulnerable to reinfection after variable time. Experimental infection in volunteers suggest that susceptibility to infection, clinical illness, its severity and recovery are influenced by strain variation, length of time since the previous URI and the presence of specific antibodies (Callow, 1985; Reed, 1984).

Epidemiology: Serosurvey based studies have identified CoV in North and South America, Europe and Asia (Larson et al., 1980; Hasony and Macnaughton, 1982; Schmidt et al., 1986). CoV appears to be likely to cause respiratory infections at extremes of age as well as patients with chronic obstructive pulmonary disease (COPD). In a day care center for older persons CoV infection is an equally important cause of acute respiratory illness as HRSV and influenza (Falsey et al., 1995a), the prototype 229 E observed to be readily co-circulated with HRSV and influenza in this environment.

Clinical illness: Clinical illness associated with human CoV is most typically URI. Symptoms of pharyngitis, rhinorrhea, headache, malaise and cough are common.
LRTI is more common in CoV than rhinoviruses. Nosocomial infections are common. Human CoV respiratory infection is associated with asthma in both children and adult.

**Diagnosis:** Previously, serodiagnosis was the only method, which was slow and insensitive. Culture of clinical specimen is difficult hence results in false negative results. Other methods include RNA-RNA hybridization, western blot (WB) and RT-PCR, which are recently attempted only in isolated cases. WB using recombinant coronaviral protein N and S are more sensitive in pediatric population. Direct RNA hybridization using nasal secretions might be adaptable for screening of 229E (Myint et al., 1989).

**Therapy:** No antiviral treatment is available today. Proteinase inhibitors like leupeptin, cystatin C and E 64 have been shown to inhibit virus replication in cyto. Use of interferon-α-2b and interferon-β appears to be ineffective in natural infection.

**Sever Acute Respiratory Syndrom (SARS) Coronavirus:** In late 2002, cases of life threatening respiratory disease with no identifiable cause were reported from Guangdong province, China. They were followed by 32 other countries, which included, Hongkong, Phillipines, Singapore, Thiland, Vietnam, Indonesia, United Kingdom (UK) and United States (US). The outbreak was originated from single ill healthcare worker and then spread to household members and many other health care workers. In early 2003 the syndrome was designated ‘Severe Acute Respiratory Syndrome’ (SARS). Electron microscopic pictures from cell culture and respiratory specimens from SARS patients showed corona like particles. World Health Organization (WHO) named this new CoV as SARS coronavirus. This novel CoV was associated with this outbreak and the evidence indicated that this virus has an etiologic role in the SARS. Partial sequencing of SARS corona virus polymerase gene indicated that this virus is genetically distinct from existing major corona virus antigenic groups (Holmes, 2003; Marra et al., 2003).

SARS quickly spread to become a global menace leaving more than 800 dead from 8000 infection in 32 countries. About 347 of fatality and 5326 of the infections were recorded in China (Thomas, 2003). SARS is a severe, rapidly spreading new disease in humans. This appeared to be first severe and early transmissible new disease in the 21st century. SARS appeared to spread most commonly by close person-to-person contact involving exposure to infectious droplets and possibly by direct contact with infected body fluids. The signs and symptoms of the disease include flu like illness; in some cases this is followed by bilateral pneumonia, in some cases progressing to acute
respiratory disease requiring assisted breathing on a respirator. In some, death resulted from respiratory failure.

Treatment is supportive and diagnostic procedures include virus isolation in African green monkey kidney (VERO) cells from respiratory specimens or blood of SARS patient. Other methods include EM, PCR, and detection of immunoglobulin M/A (IgM/IgA) from patient’s serum in ELISA or using infected VERO cells. SARS outbreak was brought under the control using three types of interventions (Dwosh et al., 2003) i.e. infection control measures, organizational intervention and development of SARS assessment and treatment unit. Infection control measures included, isolation of patient in a private room (with negative pressure if possible), voluntary home quarantine for staff, patients and visitors, stringent hand washing in all hospital areas, wearing of gloves, gowns and N95 masks by all staff and visitors of the hospital, use of eye protection in patient care areas, elimination of nebulized medications and moratorium on non-invasive ventilation. Organizational interventions consisted of closing the emergency department, suspension of elective surgery, prevention of patient transfers between the facilities, cancellation of out patient diagnostic procedures and restriction of hospital visitors. SARS development and treatment unit consisted of a dedicated unit on a separate ward, rooms retrofitted with externally exhausted high efficiency particulate air (HEPA) filters and full haemodynamic monitoring and ventilator support for critically ill patients with SARS.

1.2.5 Rhinovirus

There are 100 serotypes of rhinoviruses that cause colds in addition to the several strains of coronaviruses and thus likelihood of any successful vaccine substantially reducing the burden of common cold is not easily possible. Homo and heterotypic antibodies do not protect from reinfection. Available antiviral agents have not resulted in clinically important lessening of cold severity or duration, during natural colds.

Acute febrile upper respiratory diseases caused by rhinoviruses are defined "common cold", which is the most frequent infectious illness of humans. Young children were most susceptible to the infection; increasing susceptibility is associated with decreasing age with four to six colds per year in young children and two to four colds per year in adults. Colds are an important cause of discomfort, occasional complication and economic loss worldwide but there is no unequivocal data for lower respiratory tract.
replication of rhinoviruses in natural infection. The viral etiologies of colds are notably rhinoviruses (RV) and coronaviruses.

Human RV belongs to family *Picornaviridae*. RV14 is the major and RVIa is minor group of RV. There is no common group antigen. Virus infects and replicates in the ciliated epithelial cell lining of nose. Epithelial cells are shed during infection and the amount of virus shedding relates with severity of clinical illness (Douglas, 1966). In natural infection, inflammation and edema of sinus is associated with rhinovirus infection.

**Clinical illness:** Incubation period is up to 1-4 days followed by pharyngitis, sneezing, nasal obstruction and rhinorrhea, which predominate. Generalized complaints of chills, fever, headache, myelgia, chest pain and anorexia are also defined in few patients. Otitis media in children and sinusitis at all ages is also characteristic of the virus.

**Diagnosis:** Isolation of rhinovirus from respiratory secretions in (diploid strains of human embryonic lung cells) WI-38 and MRC-5 cells is one of the methods of diagnosis. Diploid fetal tonsil cells and HeLa cells can also be used but they vary in sensitivity. RT-PCR for the detection of rhinoviral nucleic acid is sensitive. Fluorescent antibody and immunoperoxidase method for detecting rhinovirus antigen is useful in experimental studies. Commercially available assays for detection of rhinovirus are not available.

**Therapy:** Steroids and interferon have no effect on rhinovirus infection. Anti-receptor (ICAM-1) MAbs block infection of major group of viruses *in vitro*. Studies in human volunteer experimental infections, high dose (100 mg) and multiple doses of MAbs were required to reduce viral titer and delay the onset of symptoms (Hayden *et al.*, 1988).

**1.2.6 Human Metapneumovirus**

The human Metapneumovirus (HMPV) recently has been isolated from the nasopharyngeal aspirates of young children in The Netherlands. Based on virological data, sequence homology and gene constellation the virus has been assigned preliminary to the family *Paramyxoviridae*, subfamily pneumovirinae and genus metapneumovirus (van den Hoogen, 2001). HMPV have been recently identified in children and adults with acute respiratory tract infections in various parts of the world viz. Canada, Australia, UK and England. HMPV strains are clearly clustered into two antigenic subgroups. At
the nucleotide level similarity between the groups was 84% to 85% as compared to within group 98% to 100% (Boivin et al., 2003).

Epidemiology: Similar to HRSV, HMPV is the respiratory pathogen with epidemic behavior and reinfection with HMPV is observed. In temperate countries HMPV infections are clustered in the period of mid March to mid April (Peret et al., 2002). Serological studies in The Netherland showed that virtually all children by the age of five years have been exposed to HMPV and that the virus has been circulated in humans for at least 50 years (van den Hoogen, 2001). In a study in Canada on hospitalized patients with ARTI, HMPV antigen was detected in 5.8% of the patients as compare to 56.7% for HRSV and 23.6% for influenza A. In children with bronchiolitis, HPMV was detected in 67%, HRSV in 84%, and influenza A in 51% of children (Boivin, 2003). A study results in Hong Kong also reported 5% patient with HMPV and HMPV was associated with 36% pneumonia, 23% asthma exacerbation and 10% acute bronchiolitis (Peiris et al., 2003).

Clinical Illness: The clinical syndrome of infected children ranges from mild respiratory problem to bronchiolitis and pneumonitis.

Diagnosis: Rapid antigen detection is currently not available. PCR is advantages for the detection of HMPV antigen. It is fastidious virus and difficult to grow in most of the cell lines, as compare to other cell lines LLCMK-2 is the sensitive cell line for HMPV and gives clear cytopathic effect (Boivin et al., 2002).

1.3 Non-Respiratory Viruses

Although most of the respiratory viruses have been documented in ALRTI, some non-respiratory viruses of the families Bunyaviridae, Herpesviridae and Picornaviridae also cause viral pneumonia in adults especially in immunocompromised hosts.

Non-respiratory viruses causing viral pneumonia in adults

- Measles virus
- Hanta virus
- Varicella zoster virus (VZV)
- Cytomegalovirus (CMV)
- Herpes simplex virus (HSV type 1)
- Human herpes virus (HHV-6)
1.3.1 Measles Virus

Measles virus is the member of the genus Morbillivirus of the family *Paramyxoviridae*. Measles occur throughout the world but the incidence, age of the patient and severity of infection vary strikingly in different geographic area. It is highly infectious childhood disease characterized by a prodrome of fever, cough, coryza and conjunctivitis followed by generalized maculopapular rash. Other than this classic measles, the virus infection is also associated with gastrointestinal, respiratory, myocardial, neurological and eye disease.

Natural infection is initiated when virus reaches epithelial cells in the oropharynx, conjunctivae or respiratory tract. The lower respiratory tract is more susceptible than nasopharynx (Robbins, 1962). The cough and coryza of measles are manifestations of intense inflammatory reactions that involve the mucosa throughout the respiratory tract. Tracheobronchitis and peribronchial interstitial pneumonia are normal features of uncomplicated measles. Pneumonitis is likely to be severe in pregnancy. Croup due to MV itself occurs in up to 20% children with measles who are younger than two years of age (Ross *et al.*, 1992). Sinusitis occurs in 2-4% patients with measles.

MV is maintained in human population only by unbroken chain of acute infections in susceptible individuals. In developed countries about 10% of cases are associated with complications. Otitis media (5-9%) and pneumonia (1-7%) are some of the most common complications. Pneumonia is more common in young children and it is most frequent life-threatening complication of measles (Norrby and Oxman, 1990). Pneumonia also accounts for more than 60% of measles associated deaths. In children, pneumonia is usually caused by bacterial super infection, but in adults, MV itself causes pneumonia (Wong and Goetz, 1993). In developing countries, measles is a devastating disease with complication rate as high as 80% in many epidemics with case fatality rate about 15-20%. Diarrhea (20%-70%) and pneumonia (20% to 80%) associated with MV infection are frequently fatal.

**Diagnosis:** Classical measles is readily diagnosed clinically where Koplik’s spots appear in nearly all patients. The aid of laboratory diagnosis is essential for measles when unexplained pneumonia or encephalitis occur without rash in immunocompromised patients. Virus isolation in cell culture from respiratory secretions, conjunctival swab,
urine as well as tissue biopsy can be done. Direct detection of measles giant cells in respiratory secretions or epithelial surface such as pharynx, nasal or buccal mucosa and conjunctiva provides rapid means of diagnosis. Testing of same specimen for measles antigen by IF or immunoenzyme staining increases the diagnostic sensitivity. In convalescent phase of disease MAb to MV N protein is useful because N protein is abundant in MV infected cells. Detection of MV RNA by direct or RT-PCR or in-situ hybridization provide sensitive tool of diagnosis. Serological diagnosis includes neutralization test (NT), CF, ELISA and IF test.

Vaccines: Vaccination has markedly reduced the worldwide incidence of measles and has eliminated endemic measles from number of countries (Orenstein et al., 2000). Measles vaccine is available in monovalent formulation, in combination with live attenuated rubella vaccine (MR) and with live attenuated rubella and mumps vaccine (MMR). Recovery from natural measles results in lifelong immunity. Administration of immunoglobulin within 72 hours of exposure usually prevents MV infection and almost always prevents clinical measles. Ig if given later up to 6 days post exposure still prevents or modifies the disease.

Therapy: There is no antiviral therapy of proven efficacy. Treatment of uncomplicated measles is symptomatic. Vitamin A is effective for treatment of measles. It reduces the morbidity and mortality of severe measles.

1.3.2 Hanta virus

Hanta virus pulmonary syndrome (HPS) is systemic disease, caused by newly discovered Hanta virus of family Bunaviridae. The recognition of syndrome was in 1993 in Four Corners area of US. Forty-eight cases of HPS were reported with overall case fatality rate of 56%. The virus is predominantly associated with specific rodent reservoirs. Virus spreads directly via aerosolization of rodent urine and feces that contain shed virus particles. A dramatic increase in the rodent population in Four Corner Area (US) occurred just prior to initial out break of HPS in 1993.

HPS has remarkable predilection for affecting healthy adults with median age of 35 years (range 11-69 years), preadolescent children get only mild illness. Nearly all patients complain of fever, chills, myalgia and headache and non-productive cough is common complaint late in prodrome. Patients with pulmonary edema, expectorate amber coloured pulmonary secretions. Dyspnea is associated with advanced disease and often signals respiratory failure.
**Diagnosis:** Early diagnosis of HPS is difficult. IgM capture ELISA is the used to quantitate acute IgM antibodies. After recovering from the illness, elevated serum IgG with clinical features, diagnose the disease. The diagnosis can also be confirmed by positive immunohistochemical staining of biopsy or autopsy tissue for Hantavirus antigen or RT-PCR of the tissue.

**Therapy:** There is no specific proven therapy for the treatment of HPS. Treatment measures are entirely supportive. The viruses of the family are sensitive to ribavirin (Viramune).

1.3.3 **Varicella Zoster Virus (VZV)**

Based on the morphology VZV is classified as a member of family Herpesviridae and the subfamily Alphaherpesvirinae. VZV has narrow host range extending to selected cell types of human and simian. They produce infection of the skin, mucous membrane, viscera and nervous system.

VZV causes varicella ‘chicken pox’ during primary infection of host. It is febrile illness characterized by generalized, pruritic vesicular rash prevalent in childhood. In classic varicella cases of childhood, cases of respiratory syndromes are unusual but varicella pneumonia is serious concern in healthy adult, especially in third trimester of pregnancy (Pugh *et al.*, 1998). Varicella pneumonia is often transient resolving completely within 24 to 48 hrs. In severe cases, interstitial pneumonitis progresses rapidly and cause respiratory failure.

1.3.4 **Cytomegalovirus (CMV)**

Assignment of the virus in Herpesviridae family was based on the morphology of the virus particle. Infection with human CMV appears to be worldwide. It is the most common cause of congenital and perinatal infection. Salivary gland virus disease of newborn is severe, often fatal illness usually affecting the salivary glands, brain, kidneys, liver and lungs. In immunocompromised hosts and in bone marrow transplant recipients the most serious manifestation of CMV infection is pneumonia. CMV infection is rarely diagnosed in immunocompetent adults however; immunosuppression due to malignancy or transplantation has been associated with increased number of cases. Among transplant recipients CMV pneumonia can be a serious complication with excess mortality (Smyth *et al.*, 1991). Diagnosis is difficult because of asymptomatic reactivation of latent virus.
1.3.5 Herpes simplex virus (HSV)

HSV is the member of family *Herpesviridae*. Like CMV it infects neonates, children and adults producing wide spectrum of disease. HSV-1 and HSV-2 are the two types.

The risk factor for HSV-1 pneumonia includes, immunosupression, solid organ transplantation and damaged respiratory epithelium. Among recipients of bone marrow transplant, HSV pneumonitis occurs in 6 to 8% of cases of interstitial pneumonia. Mortality due to HSV pneumonia in immunosuppressed patients is above 80%. HSV has been isolated from 40% of patients with acute respiratory distress syndrome. A high incidence of pulmonary HSV associated with adult respiratory distress syndrome has also been reported (Schuller *et al*., 1993).

HHV-6 has been associated with sinusitis and pneumonia in bone marrow transplant recipients (Braun *et al*., 1997).

1.3.6 Enterovirus (EV)

The EVs are so designated because of their replication in human gastroenteric tract. Poliovirus, coxsackie A/B and echovirus are some of the members. Besides neurological illness many EV infections are accompanied by nonspecific respiratory signs and symptoms, which are usually mild. Bronchiolitis and pneumonia are less commonly seen. Earlier reports indicate in EV-associated illness, 46% of patients presented with URIs, 13% with respiratory distress or apnea, 13% with pneumonia, 12% with otitis media and fewer represent with bronchiolitis, wheeze, croup and pharyngotonsilitis (Chonmaitree and Mann, 1995).

1.3.7 Epstein-Barr virus (EBV)

The EBV has been associated with acute and chronic pneumonitis in adults (Hogg and Hegele, 1995). PCR and immunohistochemistry in desquamative interstitial pneumonitis (DIP) cases have demonstrated evidence of EBV in 30% cases (Oda *et al*., 1994). Recovery is typical with rare fatalities being reported.

1.4 Human Respiratory Syncytial Virus (HRSV)

For more than four decades HRSV has been recognized as a worldwide pathogen of young children. It is the most important etiological agent of respiratory tract
disease in infants and children. The virus was first isolated in 1956 from a symptomatic laboratory chimpanzee during an outbreak resembling the common cold (Morris et al., 1956). Chanock and his co-workers in 1957 recovered a virus from infants ill with lower respiratory disease. Since that time, epidemiological studies have shown that HRSV represents the single most important cause of serious lower respiratory tract disease among infants and young children in both developing and developed countries. Clinical manifestations of HRSV infection are age dependent but in young infants and children, bronchiolitis and pneumonia are the major clinical manifestations. Older children and adult may experience reinfection as an upper respiratory tract infection (URTI) and contribute to the spread of virus to susceptible individuals (Falsey et al., 1995).

HRSV causes predictable, widespread outbreaks of illness each year with seasonal variation. Youngest infants, especially premature infants and those with bronchopulmonary dysplasia, chronic pulmonary and cardiac diseases and with immunosuppression also are at high risk for severe HRSV infection. Institutionalized adults, especially the elderly also are at the risk of complicated HRSV disease. Primary HRSV infection is also suspected as a possible cause of long-term pulmonary dysfunction, especially childhood asthma (Shaheen et al., 1994). In US alone approximately 90,000 infants are hospitalized with HRSV infection yearly at an estimated annual cost of at least 300 million dollar. The burden of HRSV infection is even greater if out patient visits for children and adults and HRSV morbidity in patients with underlying conditions is included (Hall, 1999).

1.4.1 Classification

Based on morphologic criteria, organization of genome, biologic activities of proteins and sequence relationship of encoded proteins, HRSV has been classified under the family Paramyxoviridae. As per reclassification of the Paramyxoviridae family into two sub-families paramyxovirinae and pneumovirinae in 2000 by the ICTV, HRSV comes under the subfamily pneumovirinae and genus pneumovirus. Table 2 indicates classification of the family Paramyxoviridae.

Like other members of the family Paramyxoviridae, HRSV has following features:

- The genome is single strand of negative sense RNA found exclusively in an RNase resistant helical nucleocapsid that also contains viral polymerase.
The genome is transcribed by a sequential stop restart mode, in which the polymerase is guided by cis acting signals and produces subgenomic mRNAs.

Viral replication is cytoplasmic and progeny virions acquire a lipid envelope by budding at the plasma membrane.

Virus enters into host cell, by cell surface fusion.

Table 2: Family - Paramyxoviridae

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Paramyxovirinae</th>
<th>Subfamily</th>
<th>Paramyxovirinae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genus</strong></td>
<td>Sendaiivirus (mouse parainfluenza virus type 1).</td>
<td><strong>Genus</strong></td>
<td>Human parainfluenza virus type 1 &amp; 3 virus (hPIV1/3).</td>
</tr>
<tr>
<td></td>
<td>Bovine parainfluenza virus type 3 (hPIV).</td>
<td></td>
<td>Genus Pneumovirus.</td>
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<tr>
<td></td>
<td>Sendaivirus (mouse parainfluenza virus type 1).</td>
<td></td>
<td>Human respiratory syncytial virus (hRSV).</td>
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<tr>
<td></td>
<td>Human parainfluenza virus type 1 &amp; 3 virus (hPIV1/3).</td>
<td></td>
<td>Bovine respiratory syncytial virus (bRSV).</td>
</tr>
<tr>
<td></td>
<td>Bovine parainfluenza virus type 3 (hPIV).</td>
<td></td>
<td>Pneumonia virus of mice.</td>
</tr>
<tr>
<td><strong>Genus</strong></td>
<td>Rubulavirus.</td>
<td><strong>Genus</strong></td>
<td>Avian pneumovirus (Formerly known as turkey rhinotracheitis virus).</td>
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<tr>
<td></td>
<td>Siman virus 5 (Canine PIV type 2).</td>
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<td>Human metapneumovirus. (BMPV)</td>
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<td></td>
<td>Mumps virus.</td>
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<tr>
<td></td>
<td>Newcastle disease virus (Avian paramyxovirus 1) (NDV).</td>
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<tr>
<td></td>
<td>Human parainfluenza virus type 2, 4a and 4 b. (hPIV2/4a/4b).</td>
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</tr>
<tr>
<td><strong>Genus</strong></td>
<td>Morbillivirus.</td>
<td><strong>Unclassified</strong></td>
<td>Paramyxoviruses.</td>
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<tr>
<td></td>
<td>Measles virus.</td>
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<td>Tupaia Paramyxovirus,</td>
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<tr>
<td></td>
<td>Dolphin Morbillivirus.</td>
<td></td>
<td>Hendra virus,</td>
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<tr>
<td></td>
<td>Canine distemper virus.</td>
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<td>Nipah virus.</td>
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<td>Paste-des-petits-ruminant virus.</td>
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<td></td>
<td>Phocine distemper virus.</td>
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<td></td>
<td>Rinderpest virus.</td>
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</table>

HRSV has some distinguishing characteristics, which grouped HRSV in the genus pneumovirus:

- They are only distantly related by amino acid sequence, sharing low but significant relatedness in F and L proteins and possibly a domain in N protein with other paramyxoviruses.
- Pneumovirus encodes eight or 10 mRNAs compared to 6 to 7 for paramyxoviruses.
- Pneumoviruses encode additional proteins such as NS1, NS2, M2-1 and M2-2 not found in paramyxoviruses.
They lack V, D and C protein found in various paramyxoviruses and have an unusual mucin-like G attachment protein that is distinct from HN or H protein of *Paramyxoviridae*.

Like all other paramyxoviruses, HRSV is an enveloped virus. Figure 2 shows schematic diagram of HRSV virion. HRSV appears as pleomorphic particle with diameter range from 150-300 nm. The virus has 10 viral proteins. Structural proteins include three transmembrane glycoproteins organized into virion spikes on the virus envelope. They include attachment protein (G), fusion protein (F) and small hydrophobic protein (SH). Due to F protein HRSV exhibits fusion activity. Structural proteins also include two matrix proteins M and M2, important in virion architecture. Nucleocapsid (12-15 nm) of HRSV is located within lipid envelope. Nucleocapsid of HRSV has four nucleocapsid proteins; the major nucleocapsid protein N, the phosphoprotein P, the anti-termination factor M2-1 and large polymerase sub-unit L which initiate intracellular virus replication. NS1 and NS2 are two nonstructural proteins.

![Figure 2: Schematic diagram of Human Respiratory Syncytial Virus](image)

Adapted from ‘Clinical Virology’ 2nd edition, Editor Douglas D. Richman and Frederic G. Hayden, with slight modification.

HRSV has single stranded, negative sense, non-segmented RNA genome. The genome of the virus is 15 kb in length and contains ten randomly linked genes coding for at least 10 viral proteins each by single gene although two genes M2 and G overlap (Sullender, 2000). Each gene has 3' leader (60 nucleotides) and 5' trailer sequence and it has been assumed that leader and trailer regions contain specific sequences for encapsidation, as mRNAs of paramyxoviruses are not encapsidated. They also contain sequences between the gene boundaries called inter-gene regions. Figure 3 shows map of HRSV genomic RNA. In addition to genes encoding the structural proteins it also
contains accessory genes coding some non-structural proteins. At the beginning and at
the end of each gene is conserved transcriptional control; these control regions are
essential for transcription and replication.

![Map of HRSV Genomic RNA](image)

**Figure 3: Map of HRSV Genomic RNA (Not to scale)**

1.4.2 Properties of HRSV

HRSV is very labile virus, being enveloped; it loses its infectivity with lipid
solvents and during storage at -15°C to -25°C for only several days. Viral suspension can
be preserved without complete inactivation by adding protein such as 5% to 10% normal
serum or albumin, freezing rapidly and maintaining at -70°C. The virus replicates
optimally in HeLa, HEp-2 and BS-C-1 cells whereas WI-38, KB, monkey kidney, Mv-1-
Lu and BEK cells also support growth of the virus. Peak quantities of infectious virus are
seen in the supernatant of infected cells between 2-3 days after infection. Cell free virus
appears 3-5 hrs later but 90% of the virus remains cell associated (Leine and Hamilton,
1969). Continued high multiplicity passage of HRSV generates defective interfering (DI)
particles, which decrease both cytopathic effect and the yield of infectious virus
(Treuhaft and Beem, 1982).

A wide range of animal species, including cotton rats, mice, ferrets, guinea
pigs, hamsters, marmosets, lamb and a number of non-human primates can be infected
by administration of HRSV directly into the respiratory tract. The chimpanzee alone
resembles the human in being highly susceptible to infection by contact, in developing
moderate to high levels of virus replication and in developing mild signs of disease
symptoms resembling those of mild disease in humans (Belshe et al., 1977).
1.4.3 Replication of HRSV

Like all Paramyxoviruses, replication of HRSV takes place in the cytoplasm. Virus can replicate in enucleated cells (Pennington and Pringle, 1978). Figure 4 indicates replication of paramyxoviruses. In cell culture, single cycle growth curves are generally of 14 to 30 hrs duration but they can be short, up to 10 hours for some virulent strains. The effect of viral replication on host macromolecular synthesis is quite variable, ranging from almost complete shut off late in the infection to no obvious effect.

Figure 4: Replication of paramyxoviruses

Virus adsorption and entry: The multiplication cycles do not appear to differ for each paramyxovirus, except for the great variation in the length of various phases. In the virus adsorption, cell surface receptors play a major role. Cellular receptor for HRSV seems to involve binding to glycosaminoglycans containing the disaccharide heparin sulfate and chondroitin sulfate B (Feldman et al., 1999).

Upon adsorption of virus to cellular receptor where glycoprotein G plays a major role, the viral membrane fuses with cellular plasma membrane at neutral pH found at the cell surface. The viral fusion protein F mediates the fusion and consequently viral nucleocapsid gets released in the cell cytoplasm. This mechanism needs to disrupt the contacts between nucleocapsid and M protein.
Replication/Transcription: Like all negative strand RNA viruses the genomic RNA of HRSV also serves two functions; first as a template for synthesis (transcription) of mRNAs and second as a template for synthesis of anti-genome (+) strand (replication). The viral polymerase protein complex is responsible for viral genome replication. For successful synthesis of positive strand anti-genome intermediate template, the polymerase complex disregards the termination signals interspersed at gene boundaries. Full-length progeny genomes are then copied from the anti-genome template.

Viral proteins are synthesized in the cell cytoplasm. mRNA transcripts are made in the cell cytoplasm by the viral RNA polymerase. There is no need for exogenous primers and therefore no dependence on cell nuclear function. The position of a gene relative to 3' end of the genome correlates with transcription efficiency. RNA transcription initiates at the 3' genomic promoter and copies the genes by a sequential stop-start mechanism, that yields sub-genomic RNAs. Each gene begins with a conserved 10-nucleotide gene start motif that directs initiation of transcription and encodes the 5' end of the mRNA and ends with a 12-13-nucleotide gene end motif that directs polyadenylation and release of mRNA. The HRSV mRNAs contain a virally encoded methylated 5'cap and 3' polyadenylate. Each mRNA encodes a single major viral protein except for M2 mRNA, which contains two overlapping ORFs, which are expressed as two proteins.

Virion assembly and release: Intracellular site of nucleocapsid assembly is the cytoplasm. It takes place in two steps (Kingsbury et al., 1985).

1. Association of free N subunits with genome or template RNA to form helical ribonucleoprotein (RNP) structure.
2. Association of P-L protein complex.

Assembly of virus envelope takes place at cell surface. The viral integral membrane proteins are synthesized in the ER and undergo step-wise conformational maturation before transport through the secretary pathway. Folding and conformational maturation of glycoproteins are assisted by enzymes. Only correctly folded and assembled proteins are generally transported out of the ER. In the golgi aparatus, the carbohydrate side chain may be modified and cleavage of F protein occurs in transgolgi network. Finally glycoproteins are transported to the plasma membrane.

During assembly, protein-protein interactions are very specific as cellular membrane proteins are largely excluded from the virion. Finally association of
assembled ribonucleoprotein core to the appropriate path in the plasma membrane to form a complete infectious virus particle takes place and budding takes place in polarized cells at the apical surface where M protein plays a major role.

1.4.4 Proteins of HRSV

1.4.4.1 Transmembrane proteins

The G glycoprotein: It is the first major transmembrane glycoprotein of HRSV, which serves as a viral attachment protein. It is 298 amino acid backbone structure of MW 33 kd, although mature protein is 85-90 kd. This discrepancy in molecular weight is due to post-translational O-glycosylation at the more than 70 potential glycosylation sites provided by its uniquely high serine and threonine content (Collins, 1990). This unusual glycosylation pattern is characteristic of this mucinous protein secreted by respiratory epithelium and it appears to be important for viral infectivity and antigenicity. Hydrophobic domain near the N-terminal end of G protein serves as membrane anchor, leaving C-terminal two third of the molecule as ectodomain.

The high degree of antigenic and sequence diversity of G protein ectodomain has grouped HRSV into two groups A and B. They differed in a single group specific linear epitope; the activity of this epitope appears to depend upon intra-peptide disulfide bonds (Akerlind-Stopner et al., 1990). Nucleotide and amino acid sequencing of the G protein mRNA of group A (Long strain) and group B (18537) when compared with the A2 strain of group A, 94% amino acid homology was observed in both the group A strains whereas 18537 and A2 strain shared only 53% amino acid identity. Most of the divergence was occurred in extracellular domain of G.

In addition to full size G, infected cells secrete a slightly smaller soluble form of G, which is identical to virion except transmembrane and intracytoplasmic domain (Roberts et al., 1994). Antibodies to G protein neutralize virus in vitro and immunization of G particularly protects animals from challenge although the protection is group specific (Johnson et al., 1987; Walsh et al., 1987).

Fusion protein (F): This is second major transmembrane glycoprotein. The protein is extremely hydrophobic, 514 aa in length with molecular weight 63.453 kd. The three extremely hydrophobic domains are present in the protein. The HRSV F protein directs viral penetration by fusion between the virus envelope and the host cell plasma membrane. But unlike other paramyxoviruses the HRSV F can mediate entry and syncytium formation independent of the other HRSV proteins. The precursor Fo
molecule is biologically inactive and cleavage of Fo by host cell protease to the disulfide linked subunits F1 and F2 activates the protein, rendering the molecule fusion active and permitting viral infectivity. The cleavage activation domain is at C terminus of smaller N-terminal F2 subunit. Later, in infection F protein expressed on the cell surface can mediate fusion with neighboring cells to form syncytia.

F protein is also a target for cytotoxic T lymphocytes. This protein carries several neutralizing epitopes two of which are related to fusion activity and which are highly conserved among isolates of both the virus groups (Walsh et al., 1986). Amino acid sequence identity between the both the groups (strain A2 and 18537) for F protein is 91% (Johnson and Collins, 1989). Experimental evidence suggests that F glycoprotein of HRSV is the major target of cross protective immune response. MAb and polyclonal sera raised against F protein can neutralize HRSV (Taylor et al., 1984; Walsh et al., 1987). Passive administrations of anti-F antibodies or immunization with purified F confer protection against a challenge with HRSV of two antigenic groups A and B (Routledge et al., 1988).

**Small hydrophobic protein (SH):** The third transmembrane protein is SH protein of MW 7.5 kd which exists as a multimer in infected cells. Different species of SH include SHo and SHf, which are non-glycosylated. However, SHg and SHp are glycosylated. SHo and SHp are predominant forms in the virion but the precise function of SH protein and its multiple forms is not known. SH does not carry neutralizing epitope but syncytium formation using plasmid expressing G, F and SH was most efficient when all the three proteins were present suggesting that SH enhanced the functions of one or both the other two glycoproteins (Heminway et al., 1994).

**Matrix protein:** HRSV has two membrane associated matrix proteins M1 and M2, which are thought to be important in virion assembly and membrane stabilization (Collins, 1991). Immunization of animals with vaccinia virus expressing M2 protein provides transient (<30 days) protection from challenge with either group A or B viruses. M2 protein is also a target for CTL in mouse model of HRSV infection and humans (Comers et al., 1991; Kulkarni et al., 1995).

### 1.4.4.2 Nucleocapsid proteins

**Nucleoprotein (N):** Viral replicative machinery includes the N, P and L (polymerase) proteins, which are bound tightly to viral RNA, where N protein is major nucleocapsid protein. N protein of HRSV is 391 aa in length with MW 45 kd. It is
smaller than its paramyxovirus counterparts. Like other paramyxoviruses, N protein of HRSV serves several functions of viral replication, including encapsidation of genome RNA into RNase resistant nucleocapsid, association with the P-L polymerase during transcription and replication and most likely interaction with M protein during virus assembly. The intracellular concentration of unassembled N is also thought to be a major factor controlling the relative ratio of transcription and replication. A comparison of protein sequences coupled with data obtained from protease digestion of nucleocapsid suggests the N contains two domains. The N terminal, 80% of the protein is well conserved among related viruses where as C terminal, 20% is poorly conserved. The degree of sequence identity of N protein between prototype viruses of group A and B, range up to 96% (Johnson et al., 1987). The N protein has been identified as a target for CTL (Comners et al., 1991).

**Phosphoprotein (P):** It is one of the proteins of virus replicative machinery. The protein is 241 aa length with MW approximately 33 kd. It is a major phosphorylated species in purified virus and infected cells (Lambert et al., 1988). It plays important role in the encapsidation of minigenome together with N protein. P protein also functions as polymerase co-factor and must be phosphorylated to be functional. The difference between P protein of group A and group B was observed due to variation in the size of P protein, it was 36 kd in A strains and 34 kd in B strains (Akerlind et al., 1988). Sequence comparison between the cDNA clones of the P protein gene of HRSV strains from subgroup A (A2) and B (18537) revealed 96.38% homology at nucleotide level and 99.17% at amino acid level (Lambden et al., 1985).

**Polymerase protein (L):** It is large protein of viral replicative machinery, 2165 aa in length with MW 250 kd. It is very similar in size to its paramyxovirus counterpart and contains six discrete segments that contain highly conserved residues presumably represent polymerase motif.

**Non-structural proteins (NS1) and (NS2):** NS1 (13.8 kd) and NS2 (14.5 kd) are non-structural proteins of HRSV. Both the proteins are detected in only trace amounts in the preparation of purified virions. The NS1 protein has been shown to co-precipitate with M protein whereas NS2 co-precipitates with N protein. NS1 and NS2 singly or together do not appear to have an important functional role and do not appear to participate in host immune response (Collins, 1991).
1.4.5 Antigenic groups of HRSV

Application of monoclonal antibody technology has concluded that there are two major antigenic groups of HRSV. Anderson et al., (1985) divided HRSV strains in three groups using MAbs against F, G and N protein. Where group 1 (Long strain) and group 3 (strain A2) were similar and group 2 (strain CH 18537) was distinct. Using MAbs against HRSV long strain recognizing F, G, N, M and P proteins, Mufson et al., (1985) identified two different groups. Group A included Long and A2 strain and group B included CH-18537 and 4 viruses isolated at West Virginia. The main difference was located in G proteins of isolates. Additionally, the reactivity of MAbs with P protein of isolates varied allowing the classification of viruses into two groups. Consistent with these Gimenez et al., (1986) observed P-protein mobility in SDS-PAGE of these isolates corresponded to the previous antigenic classification. Further, on the basis of MAbs against G protein, group B strains could be differentiated into two variant B1 and B2 where the size of G protein also differed (Akerlind et al., 1988). It was suggested that group B viruses are more heterogenous than group A. Difference between the two groups has led to vaccine development strategies that should provide protection against both antigenic groups.

During past three decades the two groups have been relatively stable antigenically and have co-circulated in most of the yearly epidemics studied, with group A usually predominating (Hall et al., 1990). However, prevalence of group A and B during successive years was also observed (Waris, 1991; Peret et al., 1998). Group A are often thought to be associated with more severe disease (Walsh et al., 1997), but Hornsleth et al., (1998) observed group B strains more virulent. Distribution of age of children, gender of the patient, severity of infection and proportion of hospital admission showed no difference with respect to the grouping of HRSV. Nucleotide sequencing of G protein of Long and A2 strain of group A and CH-18537 of group B HRSV revealed, G proteins of two group A viruses was very similar, where as group A and B viruses had extensive differences in the ectodomain of G protein with strictly conserved 13 amino acid region at center. Other proteins were similar although SH protein was variable (Collins et al., 1996).
1.4.6 Epidemiology

Epidemics of HRSV are local rather than national or global in spread. The virus does not show prejudice towards country or climate but does have particular preference for very young in each area of the world. Hallmarks of HRSV are:

- It is the single most important cause of hospitalization for viral respiratory tract disease in infants and young children worldwide.
- It infects the very young infant and even neonate despite the presence of transplacentally derived HRSV specific maternal serum IgG.
- Re-infection is common with reduced disease.
- It produces large yearly epidemics.

HRSV is the only viral pathogen that regularly produces outbreaks of infection each year in urban areas (Parrott et al., 1974; Mufson et al., 1973; Anderson, 1991). The outbreaks are usually sharp in onset and limited in length, lasting 2-5 months and they tend to recur annually at regular and predictable intervals. During the peak of epidemic of HRSV, other viruses with epidemic potential are usually absent from the community some times mixed infections may occur (Mufson et al., 1988).

Humans are the only recognized natural hosts of HRSV, although bovine and ovine HRSV strains are economically important causes of respiratory illness in cattle and sheep. In virtually every epidemiological investigation, HRSV is single most important cause of pneumonia and bronchiolitis under the age of 1 year, accounting for 5-40% of all pneumonia, 40%-90% bronchiolitis and 3-10% of croup, which require hospitalization (Hall and McCarthy, 1996). Bronchiolitis and pneumonia occur most frequently between age of 6 weeks and 9 months and the peak incidence of lower respiratory tract disease between the ages of 2 and 7 months, this age correspondence the diminishing titer of maternally derived antibodies (Holberg et al., 1991).

The risk of serious HRSV disease is increased by pre-maturity, young age, gender, chronic cardiac or lung disease, immunodeficiency or immunosuppression and family history of allergic disease. However, approximately three fourth of hospitalizations for HRSV disease occur in infants and children who are previously healthy (Wang et al., 1995). Lower socioeconomic status, household crowding, exposure to cigarette smoke and lack of breast-feeding have also been associated with more serious disease.

Among adults, HRSV infections are less common, and though often result in mild upper respiratory illness, rarely cause community acquired pneumonia (Dowell et
al., 1996). Elderly persons, especially institutionalized adults and persons with underlying heart and lung disease, are at high risk for developing severe HRSV infection. HRSV ranks second to influenza as a major viral pathogen in this age group (Falsey, 1998).

1.4.7 International status

HRSV is the cause of one-fifth of lower respiratory infections worldwide and it is the most common pathogen causing lower respiratory tract infections in infants. Lower respiratory infections caused by HRSV occur epidemically, and the appearance of an epidemic seems to vary with latitude, altitude and climate. HRSV epidemic has a seasonal pattern typically depend on geographic location and altitude. During these seasons the epidemics tend to appear in clusters. Although the appearance pattern of these epidemics varies from one continent to another, they usually begin in coastal regions.

In equatorial band HRSV appears to be present either year round or in the middle of the year and some increase during the dry months (Chew et al., 1998; Doraisingham et al., 1986). From this region epidemic appear temporally in a south to north direction in northern hemisphere and north to south in southern hemisphere. In countries with temperate climates HRSV epidemics occur in late fall, winter and spring (Kim et al., 1973; Orstavik et al., 1984; Hall et al., 1990; Berner et al., 2001). In addition, HRSV studies conducted in temperate climates have identified a bimodal pattern in the peaks of HRSV epidemics and hospitalization rates (Waris et al., 1991; Foy et al., 1973; Monto et al., 1974).

In tropical and semitropical climate HRSV epidemics occur equally regularly, but with different patterns of seasonality. In northern tropical areas seasonality seems to be associated with a decrease in temperature and an increase in rainfall (Weber et al., 1998; Cherian et al., 1990). Over the 3 years period of surveillance, HRSV epidemic occurred in Trinidad during the rainy season, which extended from June through December (Spence and Barratt, 1968; Sung et al., 1987), However, in a pediatric ward at Saint Pierre de la Reunion located in the Indian Ocean increasing in HRSV infection was reported during the hot and rainy season (Sapin et al., 2001). In tropical areas south to equator HRSV seasonality is associated with decrease in both temperature and rainfall (Weissenbacher et al., 1990; McAnerny et al., 1994). However, reports from Singapore
(Chew et al., 1998) and Brazil (Straliotto et al., 2001) indicate no relation between HRSV infection and relative humidity.

In developed as well as developing countries HRSV has a characteristic pattern of activity with peaks at 12 to 14 month intervals. In Chicago, it was observed that interval between successive peaks was alternatively long and short i.e. 13-16 months and 7-12 months respectively (Kim et al., 1973). In Seattle the peak varied from December to April, with 13 months interval for 3 years followed by 9 months interval (Foy et al., 1973; Glezen, 1977; Mufson et al., 1973).

Age distribution of HRSV infection in children in developed as well as developing countries is similar (Weber et al., 1998). In developed countries due to effective modern intensive care, mortality due to HRSV infection is not common, it is less than 2% among hospital admitted children. However, in children underlying cyanotic congenital heart disease, case fatality rate is as high as 37% (Walsh et al., 1997). In these countries, there are well-defined high-risk groups for the infection of HRSV, which include infants with a history of premature birth, those with bronchopulmonary dysplasia, congenital heart disease, immunosuppressed patients, institutionalized individuals and elderly people. However, European countries along with other developed countries report about 20% HRSV infection in hospitalized children (Orstavik et al., 1984).

In developing countries risk factors for HRSV disease are not defined, although crowding, indoor smoke pollution and malnutrition may play an important role in the development of more severe disease (Selwyn, 1990). Data on acute respiratory tract infections in children under 5 years of age from 10 developing countries like Argentina, Colombia, Guatemala, Kenya, Nigeria, Pakistan, Papua New Guinea, Philippines, Thailand and Uruguay showed that the most frequent cause of ALRTI was HRSV accounted for up to 70% of all cases. This rate is as high as 78% in Bangladesh, 72% in Singapore and 79% in Saudi Arabia (Huq et al., 1990, Chew et al., 1998, Bakir et al., 1998). In developing countries in hospital inpatients the mortality rate up to 7%, which is much higher than that seen in high-risk patients in developed countries (Cherian et al., 1990).

Soon after the discovery of HMPV, researchers in The Netherlands reported co-circulation of HMPV and HRSV in infants and children with respiratory tract disease. The clinical symptoms of the children infected with HMPV and HRSV were similar, ranging from URTI to severe bronchiolitis and pneumonia (van den Hoogen et al.,
2001). In Canada, when percentage of hospitalization for ALRI in children less than or equal to three years of age attributable to HMPV and HRSV was studied, HRSV was detected in 57% of 208 children, HMPV was detected in 6% and influenza A in 24% of children. Bronchiolitis was observed in 84% and pneumonitis in 25% of patients positive for HRSV (Biovin et al., 2003). Similarly a report from Germany indicated a little higher percentage of HRSV in 23.8% of 63 children as compare to 17.5% with HMPV. They also indicated three patients simultaneously infected with both HMPV and HRSV (Viazov et al., 2003).

1.4.8 National status

A striking pattern in the appearance of HRSV epidemics occur globally, epidemics almost uniformly start in coastal areas or areas surrounded by water and then progressively appear in nearby areas in the subsequent months. In India also annual outbreaks of HRSV are observed every year where the virus appears to play an important role in childhood infection. Similar to global picture, in India HRSV first appears in the coastal areas of South India and then progresses in South North direction. In South India HRSV appears in July /August with peak season during August through October, which are rainy months (Steinhoff et al., 1985; Cherian, et al.1990; John et al., 1991). In Kolkata, West Bengal, HRSV appears in August/September. It was also observed that outbreaks of HRSV infection depended not only on temperature and humidity but also on crowding, leading to increase contacts between potentially infective and susceptible individuals (Ota and Bang., 1972; Hillis et al., 1971). In New Delhi the peak HRSV activity is reported in September i.e. winter month (Patwari et al., 1996; Maitreyi et al., 2000). In Chandigarh, Agarwal et al., (1971a) reported maximum incidence of HRSV in January. Figure 5 indicates activity of HRSV reported in India.

Earlier limited studies in India indicated that HRSV has remained the single most important cause of hospitalization for viral respiratory tract disease in infants and young children less than 1 year of age. Preliminary studies at Vellore in 1981-82 indicated predominance of parainfluenza viruses among acute respiratory infections (Steinhoff et al., 1985) but later, three years systematic study was conducted at Vellore to understand the etiology of acute respiratory infection in children (John et al., 1991). Virus infection was detected in 49% and 38% of 809 children with lower and upper respiratory tract infection respectively, of which 30% infections in the age group <1 year were due to HRSV. Earlier, Subramanian et al., (1980) also observed that, in respiratory
specimens collected from 400 infants from hospitalized cases in Madras showed 25.8% isolation rate for HRSV in age groups 7-12 months.

Figure 5: HRSV activity reported in India.

Reports from India also indicate that HRSV is the major cause of bronchiolitis and pneumonia in early infancy and childhood. A study of 150 hospitalized cases at Chandigarh showed 43% of the cases with bronchiolitis and 33.6% with bronchopneumonia, were positive for HRSV infection (Agarwal et al., 1971a). A recent study in New Delhi indicated that 14% of 132 children with clinical and radiological evidence of bronchopneumonia/pneumonia had HRSV infection (Patwari et al., 1996). A study by Maitreyi et al., (2000) using 200 nasopharyngeal aspirates collected during 1995-97 at New Delhi, demonstrated presence of virus by isolation in 44.5% of the cases studied. 25% of the cases with bronchiolitis had HRSV infection and the isolation was equally distributed among group A and B.

Sero-epidemiology conducted at Chandigarh during 1970s indicated that 19.6% sera from healthy population showed presence of antibodies to HRSV with higher
titers of sera from younger age groups (Agarwal et al., 1971b). 10% of the sera collected in Pondicherry from age group 0-5 years showed presence of antibodies to HRSV when tested using complement fixation test (Madhavan et al., 1974). Serological studies in Madras indicated 27.5% of sera from children below 10 years were positive for HRSV (Subramanian et al., 1980).

Studies on HRSV were conducted in Pune at National Institute of Virology between the years 1989-1995. Specimens collected from pediatric cases with respiratory infections during the year 1989-90 were tested in enzyme immunoassay employing HRSV EIA kit and five percent of the specimens were positive for HRSV. Twelve throat/nasal swab specimens yielded HRSV during the years 1991-95 (Wkly Epid Rec., 1996). Nucleotide sequencing of G gene of seven of these isolates revealed that all the seven isolates belonged to group A of HRSV and genotyped as, two isolates each of GA2, GA3, GA5 and one was GA7 (NIV Annual Report, 2001-2002).

1.4.9 Transmission of HRSV
Outbreaks of HRSV tend to spread slowly rather than explosively like influenza. It is most efficiently transmitted by aerosol droplets and also by direct contact with secretions of infected person and by large particles and fomites. Despite its thermolability, HRSV is stable on various surfaces sufficiently long, to allow dissemination from the environment. HRSV is generally introduced into the home by school-aged children with upper respiratory symptoms and rapidly spread to other members of the family including newborns and adults. Hospitalized infants generally shed virus for 7 days, during re-infection the viral shedding is brief.

1.4.10 Reinfection
Reinfection of HRSV occurs in all age groups. HRSV infection in adults always represents reinfection. It is generally less severe and reflects incomplete immunity of an individual to the virus. Underlying cardiopulmonary disease, immunocompromised adult and frail elderly are at significant risk of HRSV reinfection. Mufson, (1987) assessed the group characteristic of the viruses. Reinfection with heterologous group is favored because it can partially evade previously induced immunity. Waris, (1991) observed that children older than 6 months during their first HRSV infection were more resistant to homologous than heterologous group reinfection.
1.4.11 Nosocomial infection

In case of hospitalized children when HRSV is prevalent in community
nosocomial infections with HRSV commonly cause serious illness in children less than
12 months of age and up to 40% of infants develop pneumonia. Hall et al., (1975a)
correlated the risk of getting nosocomial infection directly with the length of
hospitalization. Hospital staff are responsible for most of the transmission of HRSV in
the hospital hence strict hand washing and use of gloves for handling infectious
secretions is indicated to reduce the spread of HRSV. These infections are also related to
the design of the hospital ward and age of the patients.

1.4.12 Clinical manifestations

Primary infection: Asymptomatic HRSV infection is considered to be rare.
Illness almost always begins with upper respiratory symptoms. Nasal congestion with
mucoid discharge is present. Pharyngitis and fever (38°C-40°C) are common,
accompanied by systemic symptom of irritability and poor feeding. After several days
with URI involvement, signs of LRI become prominent. Cough is common, dyspnea,
tachypnea and signs of respiratory distress may develop. Virtually all patients with
primary HRSV infection have some degree of hypoxemia.

Clinical syndromes caused by HRSV: HRSV is the major cause of
bronchiolitis and pneumonia in early infancy and childhood. These two syndromes
represent continuum of LRTI.

Bronchiolitis: It is the most common clinical manifestation of LRTI. It is
estimated that about 43% to 90% of all cases of childhood bronchiolitis are due to
HRSV. There is seasonal occurrence of bronchiolitis simultaneous with annual HRSV
epidemic. The course of disease usually results in improvement of clinical signs in 3-4
days, despite viral shedding for 2-3 weeks. Signs of bronchiolitis include wheezing and
hyperaeration of lung, rales and ronchi are frequently detected. Hypoximic and dyspnic
children may have chest wall retraction (Figure 6). Pharyngitis and conjunctivitis are
often associated with the syndrome. Patient may continue to have a degree of hypoxemia
several weeks after the acute illness. The pulmonary function abnormalities persist for
months or years after an episode of acute bronchiolitis. Children who had clinical and
radiological evidence of bronchiolitis at young age and who remained symptom free
during 10 years can have residual parenchymal and airway disease.
Pneumonia: HRSV has been implicated in causing 5-40% of pneumonia in infants and young children admitted to hospital. During HRSV epidemic the occurrence of pneumonia parallels that of bronchiolitis. Low-grade fever at the time of hospitalization is accompanied with cough, rhinitis, dyspnea, and wheezing. Pneumonia can be distinguished from bronchiolitis on the basis of chest roentgenogram. In pneumonia multiple areas of consolidation are observed and hyperinflation of the lung is frequently found.

Sudden infant death syndrome (SIDS): HRSV has been detected in the lungs of children dying suddenly and unexpectedly. However, no significant association between HRSV and SID has been observed. Detection of HRSV in some SID cases likely reflects prevalence and peak incidence of both HRSV and SID.

HRSV and heart disease: The risk of severe HRSV infection and mortality in infants with congenital heart disease is significantly greater than the normal infants. The functional cardiac abnormality leads to unfavorable outcome is pulmonary hypertension. Affected children die due to irreversible hypoxia.

Otitis media: Involvement of virus in otitis media is associated with bacterial pathogen.

1.4.13 Pathogenesis of HRSV infection

In severe cases of HRSV infections immunopathology results from process of viral clearance. HRSV has an incubation period of 4 to 5 days. Virus replication initiates in nasopharynx primarily in superficial layer of respiratory epithelium reaching titer of $10^4$ to $10^6$ TCID50 per ml of nasal secretion in young infants. Virus spreads from URT to LRT via nasal secretions or cell-to-cell fusion. Even though HRSV is pneumotropic, in fatal infections of immunocompromised infants or adults, virus did spread to organs like kidney, liver and myocardium (Milner et al., 1985).
Pathology associated with fatal HRSV infection in normal infants is localized in the 75-300 μm diameter bronchioles. There is necrosis of bronchiolar epithelium, syncytium formation, loss of cells and increased production of mucus. In addition, there is infiltration of the underlying submucosa with mononuclear cells, predominantly lymphocytes. The epithelial damage, mucus and inflammatory cells produce a plug that effectively obstructs the small airways leading to air trapping. Airway obstruction leading to ventilation perfusion mismatching and thickening of alveolar septa is thought to cause hypoxia. In typical bronchiolitis there are relatively few virus infected cells detected by immunofluorescence, which suggests that disease is primarily a result immunopathology (Ahern et al., 1970).

Asthma and Atopy: Severe HRSV infection is strongly associated with childhood asthma and repeated episodes of bronchospastic bronchitis, which can persist in adulthood. It is also known that HRSV is an important determinant of aeroallergen sensitization during first year of life. In children with HRSV associated wheezing, virus specific IgE and IgG 4 levels are increased and histamine and other mast cell products are released. This could be IL 4 mediated Th 2 cytokine which worsens lung pathology contributing into severity of illness in children undergoing primary HRSV infection.

1.4.14 Immune response to HRSV

Non-adaptive immune response: Epithelial cells of upper airway are where HRSV first encounters the host and leads to the release of parainflammatory cytokines and initiation of non-adaptive immune response. In vitro studies show that pulmonary epithelial cell lines produce IL-6, IL-8, IL-11, GM-CSF and soluble TNF receptor after HRSV infection (Noah and Becker, 1993). These cytokines have local effects on airway epithelium, which may have antiviral effect also.

HRSV poorly induces interferon, the innate antiviral. In HRSV induced disease, macrophages can influence in their role as antigen presenting cells, as a source of cytokines and their susceptibility to infection.

Adaptive Immune Response: During 5 to 7 days incubation period, virus reaches the lower respiratory tract inducing adaptive immune response by both humoral and cell mediated mechanism. Local secretory IgA is the primary humoral mediator of resistance in the upper respiratory tract and also plays an important role in resistance to reinfection, although effect is not long lasting after primary infection. Protection of the lower respiratory tract is mediated partly by serum IgG, concentration of which are
boosted with each reinfection. Serum neutralizing antibodies persist at higher titers for much longer duration than do protection in the URI compared to LRI (Prince et al., 1985).

Cellular immunity seems to play a prominent role in recovery from HRSV infection (Watt et al., 1990). CTL controls acute viral infection, directly by destroying virus-producing cells and by releasing cytokines with antiviral activity. In addition CTL are also capable of causing lung immunopathology associated with reinfection.

At birth, infants possess passively acquired neutralizing IgG antibody and within 21-28 days after birth, HRSV specific antibody declines. Glezen, (1986) has shown that higher levels of maternal antibody co-related with reduced severity of HRSV infection during first year of life. In primary infection, infants respond with secretory and serum-neutralizing antibodies against F, G and internal proteins of HRSV and are influenced by the age of patient and presence of maternal antibodies. In young infants (<8 months) serum IgG response is less than that of IgA (Murphy et al., 1986a; Murphy et al., 1986b). Multiple re-infections with growing age induce higher levels of IgA antibodies and more sustained secretory immune response. However, secretory antibodies produced by infants in response to HRSV infection often fail to neutralize the virus in vitro (McIntosh et al., 1978). IgG1 and IgG3 are predominant subclasses in children and IgG1 and IgG2 in adults during reinfection. In adult immunity to reinfection co-relates better with nasal HRSV neutralizing IgA antibodies than with serum antibodies, these antibodies are partially protective because person still gets infected and become symptomatic in presence of such antibodies. Importance of antibody has also been shown in mouse model. Mice without antibody demonstrated enhanced pulmonary histopathology and experience more severe illness than those with an intact immune response. However, human studies and animal experiments indicate that passively acquired antibody is sufficient for protection from sever LRTI (Graham et al., 1993).

### 1.4.15 Diagnosis

With wide spread use of Ribavirin, a specific antiviral therapy for HRSV infection, the rapid diagnosis of HRSV infection is increasing in importance. Currently used diagnostic methods for HRSV include:

- Detection of virus by cell culture (Arens et al., 1986).
- Detection of viral antigen by enzyme immunoassay (EIA) (Todd et al., 1995), indirect and direct immunofluorescence (Gardner & McQuillin, 1968).
Detection of viral RNA by RT-PCR (Paton et al., 1992).
Serological detection of HRSV specific IgM antibody in acute serum or demonstration of HRSV specific IgG by EIA or neutralization assays (Anderson, 1988).
Other techniques include EM to detect viral particles and radioimmunoassay to detect viral nucleocapsid (Joncos et al., 1989; Hoffman et al., 1976).

Diagnosis of HRSV is best confirmed by isolation of HRSV from culture. By definition it is 100% specific. Optimal results are obtained by using several cell lines (HEp-2, MRC-5, WE-38), but a major drawback is, in tissue culture HRSV grows very slowly, frequently taking 5 or more days before CPE is detected. Slow growth of HRSV has remained an obstacle for clinical decision-making. But now the use of shell vial for detection of viral antigen can speed diagnosis without sacrificing sensitivity (Olsen et al., 1993a). Rapid diagnostic tests clearly supplement viral culture. These techniques include MAb based IF test for HRSV (Imagen) and EIA (Abbott Test Pack RSV and Enzygnost RSV-Ag), which are now commercially available, and routinely used. The sensitivity and specificity vary with commercial kit but the results are available in minutes to hours. The specificity and sensitivity of the test is greater than 90% when the standard is culture (Kellogg, 1991). ELISA also is as sensitive as 85% and specificity is generally greater than 90%. RT-PCR is reasonably sensitive, but preliminary information does not indicate substantial advantage over rapid tests for the diagnosis of primary infection. However, it may ultimately prove to be useful in persons shedding low quantities of virus or for inadequate sample (van Milaan et al., 1994; Freymuth et al., 1995).

Serological diagnosis of HRSV is used in retrospective study for research purpose. The results are less reliable in the primary infection of young infants due to interference of maternally derived high levels of antibodies.

1.4.16 Therapy

The therapy is supportive and mostly directed at reversing physiological and inflammatory effects of infection and reduce virus shedding. In infants, supplementing low concentration of oxygen reveals hypoxia but in severe cases ventilatory support is required.

Two means of management of HRSV infections are there, namely a chemotherapeutic drug, ribavirin and a humanized monoclonal antibody for prophylaxis.
i.e. Palivizumab. Ribavirin was approved in 1986 for use in the treatment of acute HRSV infection in hospitalized infants and demonstrated improvement in clinical illness and decreased virus shedding. Antiviral ribavirin, a synthetic nucleoside is administered as a small particle aerosol into tent, mask or ventilator. The drug is given for a total of 12-20 hrs per day for 2-5 days at a reservoir concentration of 20 mg/ml (Hall et al., 1983). Recently high dose, short duration therapy i.e. 6 hrs/day at 60 mg/ml, has been suggested as an alternative to standard therapy (Englund et al., 1990).

Some investigators have questioned the practical relevance in the improvement in clinical illness, mortality, duration of hospitalization and high cost therapy approximately $3000 per infant (Wheeler et al., 1993). Hence current recommendation by the Academy of pediatrics, Committee on Infectious Disease (CID, 1996) concluded that ribavirin might be considered for use in the selected group of infants like:

- Those with complicated congenital disease, bronchopulmonary dysplasia, or cystic fibrosis or severe neurological disease.
- Healthy but premature infants or those less than 6 weeks of age.
- Those with underlying immunosuppressive disorders.
- Any infected infant with severe illness (judged by degree of hypoxia).

Palivizumab appears to be effective for immunoprophylaxis of serious infection in premature children and children suffering from bronchopulmonary dysplasia (The Impact RSV Study Group, 1998). The dose of palivizumab is 15 mg/kg of body weight per month administered intramuscularly into thigh for 5 months during HRSV epidemic season. High titered Ig administered intravenously also provides satisfactory prophylaxis (Groothuis et al., 1995).

Corticosteroids and interferon have not been proven to be effective during the course of HRSV infection (Springer et al., 1990; McIntosh, 1978).

1.4.17 Vaccine

The vaccine for HRSV is the highest priority because of the common occurrence of the virus in infant and children, disease severity and reinfection throughout the life. Antigenic variability of HRSV and occurrence of HRSV infection in preexisting immunity are the challenges for vaccine development.

In early 1960, formalin inactivated alun precipitated whole virus vaccine of HRSV was administered parenterally to infants and children. This vaccine not only failed
to protect against HRSV infection but illness in vaccinees following subsequent infection had enhanced illness and some deaths. There was more LRTI and pneumonia. Rate of hospitalization and disease severity was highest in the youngest group of children, 2-7 months at the time of immunization. The vaccine induced specific circulating antibodies, failed to protect the children who received it and also enhanced the disease severity in some of them (Kim et al., 1969). Children who experienced vaccine-enhanced illness had antibody response to F and G but lacked neutralizing and anti-fusion activity (Murphy and Walsh, 1988).

To explain the pathogenesis of this enhanced illness some theories suggested that immunogenicity of F gp was diminished due to formalin inactivation, due to which virus was free to spread by cell-to-cell fusion during subsequent infection. When sufficient viral antigen reacted with antibody, immune-complex mediated reaction occurred (Merz et al., 1980). Whereas other theory suggests that a heightened lymphocyte proliferative response in these children could be one of the reasons of enhanced pathology. Since that time novel approaches for the development of HRSV vaccine have been investigated.

**Live attenuated virus vaccine:** Mucosal immunity plays a role in immunity to HRSV hence live attenuated vaccines like temperature sensitive (ts) or cold adapted (ca) mutants were developed (Waris, 1982). Intranasal administration of live attenuated HRSV has the advantage of mimicking natural infection, hence has never shown to prime for enhanced disease (Waris et al., 1997). Although initial results were encouraging this approach was abandoned as these vaccines were either poorly immunogenic, under or over attenuated, excessively virulent or genetically unstable.

**Recombinant vaccine:** F gene of HRSV inserted into viral vector such as vaccinia or adeno were found to be promising in rodents but due to lack of immunogenicity and their low efficacy in subhuman primates did not reach clinical trials (Wertz et al., 1987). Experiments by Burkreiev et al., (1996) have suggested that entire gene of one virus can be inserted into HRSV genome without affecting replication. So it may be possible to insert the G gene of A group into group B virus parent producing chimeric virus expressing G protein of both the antigenic groups.

**Subunit vaccines:** Subunit vaccine composed of the HRSV F protein for vaccination is one more approach. The F protein of HRSV carries neutralizing and fusion inhibiting epitopes, conserved among most of the HRSV strains. Preliminary results in
previously infected high-risk children, healthy and elderly have demonstrated stimulation of neutralizing antibodies to both the groups A and B.

Another potential use of subunit vaccine is for immunization during the third trimester of pregnancy in which placental transfer of protective neutralizing antibody may provide benefit during early months of life (Englund, 1994).

1.5 Hybridoma

**Hybridoma technology:** The introduction of monoclonal antibody technology brought about a revolution in immune serology. The capacity to fuse immunized B-lymphocytes with myeloma cells in culture and subsequently to select hybridomas that secrete immunoglobulin of desired specificity has made available unlimited quantities of highly useful monoclonal antibody reagents. This was achieved in 1975 by Kohler and Milstein, who first immortalized antibody secreting lymphocytes from mouse by fusing them with mouse myeloma cells and cloned hybrid cells to produce lines of hybridoma each of which secrete one particular antibody molecule. Sendai virus mediated this fusion and hybrids were selected as described by Littlefield, (1964). It is now possible to produce unlimited quantities of homogenous and specific antibodies even if the immunizing antigen is not pure.

Wide range of hybridomas is available now, i.e. Murine hybridoma (Kohler and Milstein, 1975), Murine-Human (Schlom et al., 1980), Human-Human (Olsson and Kaplan, 1980). Milstein and Cuello (1983) reported the production of bi-specific antibodies from hybrid hybridomas. They achieved this by fusing two cells, each producing antibody of predefined specificity. These bi-specific monoclonal antibodies are structurally bivalent but exhibit functional univalency.

**Production of hybridoma:** The monoclonal antibody has advantages of defined specificity, homogeneity and reproducibility. Unlimited amount of pure antibodies can be made to impure antigen, expanding MAbs in cell culture or grown in mouse ascites. Using MAb one can detect components in a mixture that are present in small quantities and not detectable by conventional anti-sera.

The steps involved in hybridoma production are outlined in Figure 7. The procedure essentially involves; immunization of animal, cell fusion, selection of hybrid cells, cloning of antibody positive cells and expansion of antibody secreting clones (Goding, 1980).
Immunization leads to expansion of the desired clones and hence the chance of obtaining relevant hybrids increases. Activated B cells have a higher tendency to fuse with myeloma cells during fusion procedure. Immunization causes B cells to divide and differentiate and hence increases efficiency of fusion. Protocols for immunization of animals vary widely. The first injection of antigen should be given in a highly aggregated form, because soluble monomeric proteins in the natural state are poorly immunogenic. Subsequent injection may be either soluble or aggregated.
For water soluble antigen the use of adjuvant is essential. Adjuvants probably induce slow and prolonged release of antigen in a highly aggregated form together with pharmacologically active substance (Chedid et al., 1976). If the antigen is of very low molecular weight or is poorly immunogenic for other reason, strong responses may be elicited by coupling it to an immunogenic carrier molecule. Currently three methods of immunization are in practice viz. *in vivo*, *in vitro* and intrasplenic.

**In vivo immunization:** The route and frequency of doses depends on the species to be immunized. The intensity of immune response depends on the route of inoculation such as IP, IV, SC and ID. When inoculated through IV route, very little antigen is involved in the induction of immune response and the rest is removed from the body. When injected into the skin the antigen is filtered by the regional lymph nodes where most of it is taken up either by macrophages or dendritic reticular cells. The remaining antigen from the blood is taken to spleen, liver, kidney and other organs of reticular endothelial system (RES) (Goding, 1983). Only the limitation is time required for the *in vivo* immunizations (1-2 month) and amount of antigen required.

**In vitro immunization:** Time required for the *in vitro* immunization is very less. Lymphocytes are cultured in flask and purified soluble antigen with N-acetyl muramyl L-alanyl-D-isoglutamine or thymocyte conditioned medium is added (Boss, 1986; Reading, 1986) and cells are used for fusion 5 days post immunization. Nanogram quantities of antigen or labile antigen can be used for immunization.

**Intrasplenic immunization:** It is very common, when antigen is in small quantities. Antigen is injected directly in spleen by anesthetizing the animal. 20 µg/100 µl of soluble antigen or 2.5 x 10^6 cells/100 µl of cell suspension is injected and spleen is used 3 days post immunization (Spitz, 1986). Due to single dose of antigen nonspecific expansion of activated B cells is avoided.

### 1.5.2 Cell fusion

Initially, inactivated Sendai virus was used as a fusogen, for the fusion of immunized spleen cells with myeloma cells (Okada and Tadokaro, 1962). It is now replaced by polyethylene glycol (Pontecarvo, 1975) or electro fusion (Zimmermann, 1982). In electrofusion the fusion of spleen and myeloma cells takes place due to application of an electric field.
Polyethylene glycol (PEG) is currently being used as a fusing agent for production of hybridomas. Now ready to use PEG is commercially available (Hybrimax, Sigma). Little is known about the mechanism by which PEG operates. It has been observed that anti-oxidants and/or polymerization agents from the commercial PEG are responsible for the PEG to become fusogenic. Wojciezyn et al., (1983) showed that membranes are brought together at closely opposed contact regions and lipid probes from one cell membrane to the other in the presence of both fusogenic and nonfusogenic PEG. Therefore, they assumed that the co-ordinate action of two distinct components is necessary and that the additives in the commercial PEG provide the fusion stimulus.

In an electric field the fusion process of spleen and myeloma cells consists of three steps. The first step is the cell migration by A C field called ‘dielectrophoresis’. The second step is the cell alignment with cell-to-cell close contact called ‘pearl chain’ and the last step is actual fusion of cytoplasm under DC pulses.

Fusion of antibody secreting cells i.e. spleen cells and myeloma cells gives raise to different types of hybrids viz. a) myeloma cells-myeloma cell b) spleen cells-spleen cells c) myeloma cell-spleen cells.

Hence to choose the myeloma-spleen cells hybrid from the culture, ‘HAT selection’ system is applied.

1.5.3 Principal of HAT selection

Littlefield, (1964) devised a method to select only those myeloma-spleen cells, which have successfully fused. The system depends on the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) from the cells. Normal cells are capable of de novo synthesis of purines. These cells in presence of HGPRT can synthesize purine by salvage pathway.

Fusion of HGPRT

myeloma cells with HGPRT

myeloma cells lead to formation of HGPRT

hybrids. These hybrids are grown in medium containing hypoxanthine, aminopterine and thymidine (HAT medium). In presence of aminopterine which blocks de novo synthesis of purines and methylation of deoxyuridilate, only HGPRT

cells can proliferate. These cells synthesize purines by the salvage pathway where hypoxanthine and guanine converted to guanosine monophosphate in presence of enzyme HGPRT. Supplementing these cells with thymidine bypasses the necessity for the methylation of thymidylate. During this selection myeloma-myeloma hybrids, which are HGPRT

die. Also hybrids formed from spleen-spleen fusion do not proliferate since they have limited
cell growth in vitro and only myeloma-spleen hybrids survive. This procedure of selection is called HAT selection system.

Selection of HGPRT<sup>−</sup> myeloma cells can be done using 8-azaguanines in growth medium. 8-azaguanine is an anti metabolite and analogue of purine (Guanine). When it is incorporated into HGPRT<sup>−</sup> cell DNA, it interferes with normal cell growth, leading to cell death. HGPRT<sup>+</sup> cells incorporate 8-azaguanine into cell DNA and die and only HGPRT<sup>+</sup> myeloma cells survive in presence of 8-azaguanine in the medium.

The HAT selection system is used for all types of hybridomas i.e. mouse-mouse, mouse-human, rat-mouse etc. After expansion of hybridoma cells to sufficient number, they can be screened for the antibody secretion.

1.5.4 Cloning

Feeder cells or conditioned medium of some sort are absolutely essential for cloning of hybridomas as they help in a situation where low cell densities are employed. In murine hybridoma, spleen cells, macrophages and blood are used as feeder cells. Allogenic or xenogenic thymocytes can also be occasionally used (Oi and Herzenberg, 1980). If phagocytic cells such as macrophages and monocytes are used they will be helpful in cleaning up the debris of dead cells found during HAT selection. Conditioned medium from almost any actively growing mouse cell line may also be used.

Once hybrids are visible in the plates, the culture supernatant is assayed for presence of antigen specific antibody secreting activity in a variety of tests such as RIA, ELISA and WB (Catt and Tregear, 1967; Engvall and Pesce, 1978; Hawkes, 1986). IF test is also frequently used method when the antigen is cell associated. Antibody secreting hybrid is then subjected to cloning. Cloning of hybrids ensures the monoclonality of the antibodies. Early after the fusion, fused cells carry a tetraploid number of chromosomes hence the cells require more energy for DNA replication. Hence, hybrid cells tend to shed chromosome and become relatively stable and divide faster. Among the chromosome they loose may be one coding for antibody required. In the culture of positive or secretor hybrid, non-secretors develop due to this phenomenon and tend to overgrow and dominate the culture. To avoid this cloning is essential. Hybridoma should be cloned twice to make certain that each is true clone. Three different techniques of cloning are,

**Cloning in soft agar:** The method involves use of semisolid agar (Caffino et al., 1972). Cells are separated in soft agar permitting clones to grow in distinct sites. The
clones are then re-cultured in liquid media to facilitate assessment of antibody production.

**Cloning using fluoresceine activated cell sorter:** Parkes et al., (1979) have used the fluorescence activated cell sorter (FACS) to select positive hybrids and clones. The single cell deposition accessory of flow-cytometer allows the FACS to plate a single cell in a well of microtiter plate.

**Cloning by limiting dilution method:** This is most widely used, simple and convenient method of cloning (Lefkovitz and Waldman, 1979). The method relies on diluting cells out to a stage where there is statistically only one viable cell per well in the culture plate.

**1.5.5 Expansion**

Antibody secreting clones obtained from a single cell are then grown in larger cultures for larger scale antibody production or freezing. As soon as there are around $10^7$ cells, a subset is frozen in liquid nitrogen to safeguard the clones. Depending upon the clone, antibody level in the culture supernatant range from 5-50 μg/mL. If very large amount of antibody is needed, it is easier to grow the cells as tumors in mice (Potter and Boyce, 1962). Pristane treated mice of the same genotype as those used for immunization are used. Jones, (1990) has shown increased production of MAbs in mice primed with Freund's incomplete adjuvant. Cells are inoculated IP in the pretreated mice. Ascites develops within 10-14 days after the injection. Generally, 5-15 mg/ml antibody is present in ascitic fluid. The MAb from either tissue culture or ascitic can be used for characterization.

**1.5.6 Applications of MAbs**

Because of their high specificity and affinity, MAbs can be used in a wide range of systems. The earlier most successful use of MAbs was in the purification of alpha interferon (Secher and Burke, 1980), they were further used for the purification of an antigen from crude mixture, using MAbs immobilized on the affinity column. MAbs are also used as a tool for identifying gene product of genome DNA libraries (Young et al., 1985). MAbs can resolve a single protein from a complex mixture and can also identify a particular site on the antigen responsible for a specific function; this antibody-binding site is called epitope. MAbs help to determine relative positions of epitopes on
antigen. Due to their high specificity at epitope level, MAbs can differentiate between strains of viruses, and have been used for strain analysis. Using MAbs a disease may be studied in greater depth since many different surface antigens are exposed at varying stages of the life cycle of infectious agent (Rowe, 1980).

MAbs have wide application in diagnostic and therapeutic use. A dye molecule can be attached to MAb without affecting the high specificity of antigen antibody reactions i.e. immunolabeling. Immunolabeling of MAbs with fluorescent dye and fluorochrome has been used in study of cell bound protein by antigen detection using antigen specific MAbs or by antibody detection such as detection of antigen specific IgM antibodies using anti-IgM antibodies.

MAbs are of prime importance in therapy of viral disease such as hepatitis and some respiratory disease with viral etiology, treatment of snake venom and specific therapy of autoimmune diseases. MAbs are also used in tumor therapy to transport toxic material to tumor cells leaving neighbouring normal cells undamaged. Human MAbs are of considerable value in immunosuppression in heart and kidney transplant and bone marrow transplantation. Use of humanized MAbs is now being done in the prophylaxis of some viral diseases.

1.6 HRSV Monoclonal antibodies: An Overview

Research on HRSV has been impeded because it grows poorly in tissue culture and most experimental animals and the virion is unstable. Still, there has been considerable recent progress in HRSV molecular biology, pathogenesis, prevention and control of HRSV disease. Previously MAbs were prepared and used to identify different structural and non-structural proteins of HRSV. Now these MAbs have greatly extended the understanding of antigenic relationship and structure of HRSV. The work on MAbs is further extended to strain analysis, mapping of antigenic sites on HRSV proteins and in the diagnosis and treatment of HRSV disease in infants or patients with underlying diseases.

1.6.1 Use of HRSV MAbs in the diagnosis

MAbs against groups A and B of HRSV have been used in diagnosis of HRSV disease as MAb based tests are most sensitive techniques when compared with culture isolation or polyclonal serum. Immunofluorescence testing with MAbs to different
proteins of HRSV has been proved a very useful diagnostic technique. Earlier studies indicate MAbs to G, F and N proteins of HRSV were used in IF test for the rapid detection of HRSV from nasopharyngeal aspirate. Sensitivity of the MAb tests was approximately equal to the PAb based commercial kit or cell culture and the specificity of the test was 100% (Bell et al., 1983; Kim et al., 1983; Stott et al., 1984; Pothier et al., 1985; Freymuth et al., 1986). Consistent with these studies, Kao et al., (1984) used MAbs in IF test and observed that agreement between them and PAb tests was 98%, and in relation to culture sensitivity was 96.98%. Freke et al., (1986) also used MAbs for the detection of HRSV from nasal aspirates. They were used singly as well as in pools in IF test. This IF test was more sensitive than any other test applied and also gave immediate results. It also produced more intense staining than the any MAb alone and gave more clearly defined staining reaction than the polyclonal antiserum (Routledge et al., 1985).

The MAb based antigen capture ELISA has found to be convenient, rapid and sensitive assay for the detection of HRSV from clinical specimens and also for the detection of individual viral antigens from unpurified preparations. Obert and Bayer, (1988) used anti-N MAbs in ELISA for the rapid detection of HRSV in nasopharyngeal secretions and the results were comparable to that of commercial reagents (PAb) in IF test. However, Hendry et al., (1985) using ELISA quantitated the HRSV polypeptides in nasal secretions of the patients. It was observed that F protein was detected in all the specimens tested, G protein was detected in 47% and N protein was detected in 51% of the specimens.

Erdman and Anderson, (1990) used MAb based capture ELISA for the detection of serum antibodies. Anti F, G and N MAbs of HRSV were evaluated for their use as detector antibodies in IgG, IgA and IgM capture ELISA. Assays using N MAbs were better for the detection of IgG and IgM antibody, F assays could detect IgA responses and IgG MAb assays were less sensitive than any other assays. A mixture of N and F MAbs was complementary overall and comparable to PAb assays.

### 1.6.2 Antigenic analysis of HRSV

MAbs to G protein of HRSV have revealed two antigenic groups of HRSV, group A and group B (Anderson et al., 1985; Mufson et al., 1985; Gimenez et al., 1986). MAbs have also been used to study circulation and predominance patterns of both antigenic groups of HRSV in the epidemic seasons. Mufson et al., (1988), using a set of MAbs in enzyme immunoassay, demonstrated predominance of group A over group B
except for only one year where B was predominant during the epidemic years of 1981-1986 in Huntington, Virginia. Consistent with this, recently, Imaz et al., (2000) observed 85.9% isolation of type A and 14.1% of type B in Argentina during the year 1993 to 1996 using MAb based IF test. Co-circulation of both the types in single epidemic season or alternate seasons were observed using MAbs specific to different epitopes of HRSV by Akerlind and Norrby, (1986). However, Freymuth et al., (1991), in the study of eight consecutive epidemics in France during 1982 to 1990 using MAb based IF test observed co-circulation of both the groups and one group rarely predominated. There was gradual change in predominant group into another over a period.

Earlier reports indicate that in addition to group A and B of HRSV some antigenically distinct strains exist which cannot be grouped. Besides 53.8% isolates of group A and 45.1% of group B, Tutsumi et al., (1988) identified one strain with intermediate characteristics using G and F MAbs. Russi et al., (1989) also had similar findings in the epidemic investigation in Uruguay. They could identify some strains, which could not be typed in any of previous groups using restricted set of MAbs.

A possible structural difference between the two groups of HRSV, antigenic characteristics and size of structural proteins of group A and group B strains was analyzed using MAbs against F, N and M protein of HRSV. These MAbs reacted with all the strains tested however; anti-G MAbs reacted with only some of the B strains giving two sets of variants, B1 (lacked epitope) and B2 (had epitope). This grouping was correlated well with size of G and P proteins (Akerlind et al., 1988). Nagai et al., (1993) demonstrated two variants, Ba and Bb in B strains isolated in Japan depending upon their reactivity with anti-N antibodies raised against B strain. Similarly, among the isolates of group A and B of HRSV, Siqueira et al., (1991) recognized four variants in group A and three variants in group B, depending upon the reactivity of these strains with anti-F and anti-G MAbs in ELISA. Mufson and Stanek, (1996) first time reported naturally occurring variant of group A (A-Var) of HRSV using anti-F MAbs generated against B group. A-Var had F protein different from that of the prototype A HRSV suggesting that A-Var is distinct type A virus analogous to group B1 and B2 of HRSV.

1.6.3 Epitope mapping of HRSV proteins

MAbs have been used to map antigenic sites (Epitope) on different proteins of HRSV so far. MAbs directed against glycoproteins of HRSV were used in competitive ELISA for topological mapping of epitopes. Extensive overlapping of epitopes was
observed on the G protein of HRSV using anti-G MAbs. However, five non-overlapping antigenic sites were mapped on the F protein by anti-F MAbs (García-Barreno et al., 1989). In MAb based study Walsh et al., (1989), demonstrated three different antigenic sites on G protein of group A and two on group B, including one cross-reactive neutralization site. MAbs to this site after passive administration could reduce pulmonary virus titers of both the A and B groups in cotton rat model. Consistent with the above results Martinez et al., (1997) also mapped three types of epitopes on the G glycoprotein of human HRSV that were located in the different regions of the G protein viz, i] strain specific epitope located in the variable C-terminal third of G protein, ii] group specific epitope shared by viruses of the same antigenic group iii] conserved epitope shared by all human HRSV isolates, located in the conserved region of the G protein primary structure.

In 1986, Walsh and his co-workers identified four epitopes on the fusion protein of HRSV using set of MAbs. Two fusion inhibiting epitopes, one non-fusion inhibiting neutralizing epitope and one non-neutralizing epitope were demonstrated on the F protein of HRSV. All but latter epitopes demonstrated partial overlapping, suggesting topographical proximity. The third non-neutralizing epitope was distinct. Contrary to this Taylor et al., (1992) observed extensive overlapping of some antigenic sites on the F protein of HRSV. It was concluded that protection against HRSV infection co-related with fusion inhibition rather than neutralization titer. Beelar et al., (1989) used neutralizing MAbs, specific for F protein of A2 strain of HRSV were used to construct topological and operational map in competition binding assay (CBA). Three non-overlapping sites A, B, C and one bridge site AB was identified. Antigenic variation in F epitope was examined using clinical isolates in cross neutralization assays with these MAbs. The analysis identified constant, variable and hypervariable regions on the molecules and indicated that antigenic variation in neutralization epitope of HRSV F glycoprotein is the result of a non-cumulative genetic heterogeneity.

Antigenic and structural variation in N protein of HRSV strains was revealed using combination of MAbs and two-dimentional peptide mapping by Ward et al., (1984). Though peptide mapping of 125 I labeled purified N from seven human HRSV strains revealed extensive homology, MAbs demonstrated distinct antigenic groups using competitive RIA. Tryptic/ chymotryptic peptides supported grouping obtained by MAbs. Murry et al., (2001) developed MAbs against N protein of HRSV and used them to study the interaction between the N and P proteins of nucleocapsid. MAbs detected a region of
aa 16-30 in the C-terminal end of N protein which interacts with P protein in the formation of nucleoprotein complex.

The epitopes of 10 MAbs directed against the P protein of HRSV (Long strain) were mapped onto the protein primary structure by testing their reactivity with protein fragments and synthetic peptides. Seven epitopes were clustered in N-terminal end; two other located in the central region and one epitope was mapped in the C-terminal end of P molecule. The location of epitopes in the P protein co-related with other properties, such as antigen binding competition and immunofluorescence staining. MAbs recognizing the N-terminal region revealed the presence of cytoplasmic inclusion, while others gave mainly a diffuse cytoplasmic staining (Garcia et al., 1993a).

1.6.4 Use of MAbs in the prophylaxis

Since discovery of HRSV in 1950s efforts have been ongoing to develop a safe and effective vaccine. Now passive immunoprophylaxis by HRSV immune globulin or Palivizumab presents a viable alternative to active immunization. Weltzin et al., (1994) reported use of IgA MAbs to F protein of HRSV HNK 20 strain for the passive immunization in mice. These MAbs when given intramuscularly 24 hrs prior to HRSV challenge reduced virus titer in lung by nearly 100 fold and could protect against both upper and lower RTI caused by HRSV. In 1996, it was observed that the same antibody when administered in a concentration of 0.5 mg/kg developed HRSV neutralizing antibodies in serum in absence of detectable virus replication. However, later it was found that topical and parenteral administration of IgA MAb (F) was no more effective than IgG MAb of the same specificity when given prior to infection (Fisher et al., 1999).

Palivizumab is genetically engineered humanized monoclonal antibody to F protein of HRSV. Tempest et al., (1991) modified the human MAb. They transferred the complimentary determining regions from murine neutralizing MAb to the fusion protein of HRSV to a human IgG1 MAb. This reshaped human MAb i.e. Palivizumab cross-reacted with all clinical isolates of HRSV, prevented disease and cured mice when administered four days after infection. In phase I and II multicenter, randomized, double blind, placebo controlled, dose escalation human trials it was found that MEDI-493 (Palivizumab) was safe, well tolerated in high-risk pediatric population when given 10 or 15 mg/kg every 30 days to the pediatric patient at high risk for serious HRSV disease (Subramanian et al., 1998). In 1998, Palivizumab were granted FDA approval (Food and drug administration) for prophylaxis of high-risk children in United States and in Europe.
in 1999 (Simoes and Groothuis, 2002; Simoes, 2002). It is now approved for use in more than 45 countries. The efficacy and safety of palivizumab continue to be supported by both clinical trials and outcome data. Intramuscular administration of Palivizumab for the prevention of severe HRSV respiratory disease in high-risk infants and young children are observed to be effective but the high cost of palivizumab was the main factor for its restricted use.