REVIEW

PREVENTION OF RESPIRATORY SYNCYTIAL VIRUS INFECTIONS

Lucia Ferro Bricks

RHCFAP/3041

BRICKS LF - Prevention of respiratory syncytial virus infections. **Rev. Hosp. Clín. Fac. Med. S. Paulo 56**(3):79-90, 2001.

Respiratory syncytial virus is the most important cause of viral lower respiratory illness in infants and children worldwide. By the age of 2 years, nearly every child has become infected with respiratory syncytial virus and re-infections are common throughout life. Most infections are mild and can be managed at home, but this virus causes serious diseases in preterm children, especially those with bronchopulmonary dysplasia. Respiratory syncytial virus has also been recognized as an important pathogen in people with immunossupressive and other underlying medical problems and institutionalizated elderly, causing thousands of hospitalizations and deaths every year. The burden of these infections makes the development of vaccines for respiratory syncytial virus highly desirable, but the insuccess of a respiratory syncytial virus formalin-inactivated vaccine hampered the progress in this field. To date, there is no vaccine available for preventing respiratory syncytial virus infections, however, in the last years, there has been much progress in the understanding of immunology and immunopathologic mechanisms of respiratory syncytial virus diseases, which has allowed the development of new strategies for passive and active prophylaxis. In this article, the author presents a review about novel approaches to the prevention of respiratory syncytial virus infections, such as: passive immunization with human polyclonal intravenous immune globulin and humanized monoclonal antibodies (both already licensed for use in premature infants and children with bronchopulmonary dysplasia), and many different vaccines that are potential candidates for active immunization against respiratory syncytial virus.

DESCRIPTORS: **Respiratory syncytial virus. Monoclonal antibodies. Vaccines. Imunoglobulins. Immunization.**

The Respiratory Syncytial Virus (RSV) is considered the primary etiologic agent in acute respiratory infections in infants. Since its identification, in the mid 50's, several studies have been conducted to develop effective and safe vaccines against RSV. However, despite more than four decades of intensive research, no effective vaccine for the prevention of infections caused by this agent has been developed up to now. In the last few years, it was demonstrated that passive immunization with polyclonal and/or monoclonal specific antibodies against RSV can either prevent or attenuate the clinical manifestations of RSV infections in high-risk groups[3,13,15,19,20,22,26,34,43,44]. In this paper, a short review on the prevention of RSV infections through the use of these pharmaceutical products is presented, as well as a review on the development of new vaccines against RSV.

The agent – The RSV was first isolated from chimpanzee's coryza in 1955 and called the *chimpanzee coryza agent*[22,44]. Soon after, the virus was isolated from children with bronchiolitis and recognized as the most common cause of lower respiratory tract diseases in children all over the world. In 1957, due to its ability to infect the respiratory tract cells and induce the formation of syncytial giant cells in tissue cultures, the virus was called respiratory syncytial virus[44,51].

The RSV is a member of the *Paramyxoviridae* family of the *Pneumovirus genus.*[34] There are two primary antigenic groups (A and B) and both present several strains.

The viral genoma, formed by a single strand of RNA, codes for at least 10 distinct proteins, most of them structural proteins. There are at least two non-structural proteins, the functions of which are still unkonwn[22,44,51]. The genoma, combined with the structural

From the Department of Pediatrics, Hospital das Clínicas, Faculty of Medicine, University of São Paulo.

proteins forms the virus core or nucleocapsid, which is enclosed by an envelope formed by different glycoproteins. The glycoproteins F and G cross the membrane and are the primary surface antigens that are the target of the host immune response. Protein F accounts for the virus penetration into cells, and for the cell membrane fusion which results in the formation of multinucleated syncytial giant cells; the protein G accounts for the virus binding to the cells[22,23,51]. Whereas protein F undergoes few changes, protein G presents greater antigenic and genetic diversity in groups A and B[11,32,50] The subtype A seems to be the most frequent, accounting for the most severe cases[12,30,56].

The aminoacid sequence of protein G, of a virus of the same group which circulates during an epidemic, can reach 20%. Like the influenza virus, the antigenic variations play a role in RSV reinfections that occur over a lifetime; however, since RSV has a non-segmented genoma, it presents less antigenic variations than the influenza virus[44].

RSV transmission occurs through direct contact with secretions from infected people or with contaminated objects[1,22]. When infected, infants and immunosuppressed individuals excrete for a long time large quantities of RSV in respiratory secretions. RSV infects the respiratory tract mucosa and the conjunctiva, and the symptoms appear following a 2 to 8 day-incubation period (most commonly 4 to 6 days)[1,44].

Clinical Manifestations –RSV replicates in the upper respiratory tract cells, causing an inflammatory process, which includes epithelium destruction, edema and increased mucus production. When the lower respiratory tract is involved, bronchiolar hyperreactivity, frequently occurs, as well as the changes already described [6,12,21-23,33].

Whereas most adolescents and adults present mild disease with upper respiratory tract symptoms (rhinorrhea and cough) and low-grade fever, RSV is considered the major etiologic agent for lower respiratory tract infections, such as bronchiolitis and pneumonia in children under two years old; however, mild and asymptomatic infections are uncommon in infants. RSV is detected in 40 to 90% of the bronchiolitis episodes and, during epidemic outbreaks, accounts for more than 80% of the cases. Half of the cases of pneumonia occurring in children under two years old are believed to be caused by RSV; this agent is identified in 10 to 30% of the cases of tracheobronchitis and, at a lower rate (3%-10%) of the cases of croup[12,23,25,30,48].

Children under two months of age, mainly preterm ones, can develop apnea and atelectasis. In their first year of life, preterm infants can present a high risk of hospital readmission, particularly those with low weight at birth and shorter gestational period[1,51,61]. Besides preterm infants, children with chronic pulmonary disturbances and congenital heart disease present a greater risk of lower respiratory tract complications. The risk of hospital admission due to chronic respiratory disease was estimated in 12%, 17% and 28%, respectively, for preterm infants without bronchopulmonary disease, with mild bronchopulmonary disease and moderate to severe bronchopulmonary disease[39].

RSV has been recognized as a major cause of morbidity and mortality in people presenting immune deficiency associated with malignancies, chemotherapy or organ transplantation and has accounted for up to 10% of hospitalizations for lower respiratory tract infections in the elderly in the winter season[12,30,34,51].

RSV epidemics frequently occur in a hospital environment, where the virus is introduced by adults who generally present either asymptomatic or mild infection.[1] When preterm infants as well as other risk groups are infected, the consequences can be devastating[55]. In a study carried out by Langley et al. (1997)[29] in nine Canadian hospitals, 6% of 1,516 hospitalized children presented hospital-acquired RSV infections, featured by the agent identification and the beginning of lower respiratory tract symptoms 72 hours after hospitalization. When compared with children with community-acquired RSV infection, those infected in the hospital environment had longer hospital stays (10 *versus* 5 days) and a higher rate of mortality (4.4% *versus* 0.42%).

RSV frequently causes bronchiolar hyperreactivity both in children and in adults[11,21,22,33]. Gross et al. (2000)[21] have recently demonstrated that the rate of RSV infection in children under one year of age admitted to hospital for chronic obstructive respiratory disease was 34.3% (74/216), whereas in the control group, which involved 133 children hospitalized for other reasons, RSV was detected in only 15% (20/133). In this study, the children admitted to hospital for CRD (chronic respiratory disease) or for other reasons presented no differences regarding risk factors for RSV infections, showing that RSV plays a major role in obstructive respiratory disease in infants[21].

In the last 20 years, a clear correlation has been observed between severe cases of bronchiolitis and the occurrence of changes in pulmonary function in childhood. Several studies have proven that, when compared with the control groups, the children presenting severe lower respiratory tract impairment following RSV infection, have, when they are six year old, reduced expiratory flow, even without recurrent attacks of wheezing. However, it remains to be clarified if RSV causes subsequent reduction of the pulmonary function (cause-effect relationship) or if individuals who already present impaired pulmonary function (atopia, bronchial hyperreactivity or reduced airway diameter) have a greater tendency to develop more severe events after RSV infections (causal relationship)[33].

Therapy for RSV Infections

The management of RSV infections is basically supportive, including diet care, oral hydration and ventilation support[51]. The antiviral therapy is restricted to the use of aerosol ribavirine[1,44].

Ribavirine is a virustatic drug that should be used early, since the greatest activity of RSV occurs up to three days after the beginning of the lower respiratory tract symptoms. Although it is a low toxicity drug that has been shown in several studies to be much more effective than placebo regarding the need of oxygen supplementation, mechanical ventilation and number of days of hospitalization, controversies still remain regarding its use, since this drug has not shown to be effective in some studies. There are doubts concerning the cost/benefit ratio of its use and the effectiveness of ribavirine in the prevention of late pulmonary sequellae following RSV infections[30,32,47].

Epidemiology of RSV Infections

Humans are the unique sources of infection. RSV is highly contagious and its transmission occurs through direct, or close, contact with contaminated secretions, droplets or fomites. It is important to stress that the virus can survive in contaminated hands for more than a half hour, being common the self-inoculation infections. The virus can survive for several hours in the surface of contaminated objects[1].

RSV presents a highly predictable infection pattern, since the beginning of outbreaks is anticipated by the increased number of hospitalized infants for bronchiolitis and/or pneumonia[23].

In temperate climate countries, the outbreaks occur in the winter season, from the end of October up to the beginning of the spring[52] and, in tropical climate countries, they occur during the rainy season[51].

It is estimated that more than 95% of children are infected with RSV up to the age of two, and more than half are reinfected every year[34]. RSV infections and reinfections are common at all ages, and a previous infection does not protect against further infection, even in the following years[51,57].

About 15% to 22% of RSV infected children under one year old present lower respiratory tract involvement and 0.5% to 2% require hospitalization[34,57]. The rate of mortality among hospitalized children range from 0% to 5.2%[44].

It is estimated that RSV causes approximately 84,000 to 144,000 hospital admissions of children under five years old[49], and 4,500 deaths each year only in the United States[26,31,43]. In late 80's, the annual cost of hospitalization for RSV in the U.S. was estimated to surpass 300 million dollars, without considering the social burden caused by work and school absenteeism.[44,51]

The outbreaks duration varies from one year to another, but on average, the outbreaks have a 22-week duration. Both RSV groups (A and B) circulate during the same epidemic period, but the rate of episodes attributed to group A and B virus varies from one year to another, and from one site to another; in addition, since there are different strains of virus A and B, outbreaks caused by genetically distinct strains can follow an outbreak caused by a single strain[12,23,25,56].

Approximately, 50% of the children are infected during RSV seasonal outbreaks[43]. In Houston (USA), the rate of infection was 68.8 per 100 children under one year old and 82,6 per 100 children under two years old; in Sweden, seroepidemiological surveys revealed that 87% of 18-month old children, and almost all children over three years old, have already been infected with RSV[20]. Most infections occur between six weeks and six months of age[1,48]. The mean age of the hospitalized children is 3 months, but among the children seen at outpatient clinics and private offices the mean age is higher. From 100 children under one year of age, 22 present lower respiratory tract disturbances, whereas from 100 children between one and two years of age, 13 present lower respiratory tract impairment[23]. Although the infections are much more common and severe in young infants, many school children present symptomatic infections caused by RSV[51].

In Brazil, as in other countries, RSV frequently circulates in the same season as the influenza virus[22,27,35,36]. Several studies have demonstrated that RSV is the primary virus isolated from children with severe respiratory disease, particularly in fall and winter seasons [7,10,12,36]. The infections are very common in infants and the prevalence in serum increases as they grow up. Cox et al. (1998)[7] observed in a seroepidemiological survey that the maternal antibodies against RSV persist on average for 3.3 months. At 3 years old, 90% of children present antibodies against RSV, whereas at 5 years old, such percentages reach 100% [7].

In São Paulo, it was observed that RSV was the most frequently isolated virus from children hospitalized for acute respiratory disturbances, from April to July 1996, in the *Hospital Universitário of São Paulo University*. From 126 children studied, respiratory viruses were isolated from 71 (56.4%). RSV was the most frequent virus isolated from 66 cases (52.4% of the children hospitalized for respiratory disturbances); this virus was detected in most cases of bronchiolitis (84%) and in almost half of the pneumonia cases (46%). The other viruses isolated were the adenovirus (4) and the influenza virus (1). The median age of the children from whose secretions the virus RSV was isolated was 3 months, inferior to that observed in the cases from which other respiratory viruses were isolated (13 months). When compared to other respiratory viruses, RSV was associated with a greater incidence of wheezing

episodes (79% *versus* 33%). In the same period, RSV was isolated from only two of the 75 children hospitalized for non-respiratory tract-related disturbances (control group), suggesting that when infected by RSV, the young children normally present symptoms[35].

Groups of Risk For Severe RSV Infections

RSV is responsible for the annual outbreaks of bronchiolitis and pneumonia which affect typically the children under six months of age. In a study conducted in the 70's in Houston (USA), Glezen et al. (1981)[16] observed that, among children from low-income families, the risk of hospitalization for infections caused by RSV was 5 out of 1,000 live born babies. More than 80% of the hospitalized children were under six months of age and the risk of hospitalization was higher among those who were born six months before RSV seasonal outbreaks. The antibody titer in the serum of 65 children with confirmed RSV infection was significantly lower than that observed in 575 children randomly selected, born in the same period[16].

In two large studies carried out in Canada, it was observed that the groups at higher risk of severe RSV infections were the preterm infants, children with bronchopulmonary dysplasia (BPD) and those with congenital heart disease (CHD)[37.55]. In both studies, more than 25% of preterm infants hospitalized for RSV infection required ICU care and 15% to 18% required ventilation support. The mortality rates were also much higher in preterm infants (0-3.3%), children with BPD (3.5-5%) and CHD (3.4-5.2%), compared to those observed in normal children (<0.3%)[44].

The major risk factors for severe RSV infections are listed in table 1[9,13,16,29,43,55].

Studies involving children under five years old from several developing countries revealed that RSV was the primary causative agent of lower respiratory tract infections, and that factors such as low social status, malnutrition, living in crowded environments (two or more individuals sharing the same bedroom, nursery attendance), birth within the previous six months from the beginning of RSV seasonal outbreaks, the mothers' low educational level and the domestic exposure to cigarette smoke predispose to more severe disease[16,34,50,51].

In the developed countries the mortality rates from RSV infections range from 0.5% to 2%, but in the developing countries, these rates reach 7%. In winter, up to 10% of the hospital admissions of elderly people are due to RSV infections and the rate of mortality achieves 10%, similar to the values observed in the infections caused by the influenza virus[51].

Immune Response to RSV Infections

The humoral response is known to play a significant role in the prevention of RSV infections. Secretory IgA protects against infections of the mucosa and the serum IgG partially protects against the infections. However, the antibodies do not provide full protection, and RSV reinfections continue to occur over a lifetime [12,57].

The cell response seems to play an essential role in the recovery from infections, considering that individuals with congenital or acquired impairment of cell-mediated immunity present more severe diseases and excrete the virus for longer periods[51]. After natural infection, normal children respond with the proliferation of cytotoxic lymphocytes specific against RSV; however, the cytotoxic lymphocytes can both help the recovery and be responsible for a more severe disease. In rats, the cytotoxic lymphocytes CD8+ are responsible for the elimination of the virus from the lungs, but paradoxically, seem to increase the severity of symptoms. The same can be observed in helper T-lymphocytes CD4+, which play a protective role and, at the same time, can increase the disease severity. There are two types of helper T-lymphocytes (Th1 and Th2), that are responsible for the production of different cytokines: the lymphocytes Th1 produce interleukin 2 (IL-2), gamma interferon and the tumor necrosis factor, whereas lymphocytes Th 2 produce the interleukins IL-4, IL-5, IL-6 and IL-3[18]. The predominant stimulation of lymphocytes Th2 are believed to be associated with more severe disease[23].

Prevention of RSV Infections

The first vaccine against RSV was developed in the 60's. It was a formalin-inactivated virus vaccine. Some children immunized with this vaccine developed a severe disease, followed by sequelae after the natural infection with RSV. Recent studies demonstrated that the inactivated vaccine produced a greater response of Th 2 lymphocytes, whereas the live virus vaccines induced a greater response of Th 1[23]. The predominant stimulation of Th 2 response

Table 1 - Major risk factors for RSV infections.

- Under one year of age, especially under six months of age
- Prematurity
- Presence of chronic pulmonary disease, particularly bronchopulmonary dysplasia and cystic fibrosis
- Presence of congenital heart disease, especially children with left-to-right shunt and pulmonary hypertension
- Cell immune deficiency; malignancies, chemotherapy, spinal cord or solid organ transplants
- Hospital admission during RSV seasonal outbreaks

and/or of cytotoxic lymphocytes is believed to have been responsible for the occurrence of more severe disease in infants vaccinated with this vaccine[28,40-42,45,50].

Several problems should be taken into account when considering the vaccination of children against RSV. The major ones are listed in table 2.

Although the immunologic characteristics of RSV are being gradually revealed, the first vaccine failure and the difficulties in obtaining immunogenic vaccines with low adverse reaction potential in young infants led to studies designed to evaluate the ways of protecting children against RSV through passive immunization [2,23,34,44,51].

Passive immunization with policlonal antibodies against RSV

Experimental studies conducted in animals, and epidemiologic findings obtained from full-term children revealed that high antibody titers against RSV's protein F (1:200 to 1:400) provide protection against pulmonary infection caused by this agent. Children with high titers of transplacental-acquired antibodies remain free from infection for longer periods of time than those presenting low antibody titers; therefore, serum antibodies can provide partial protection against RSV infection[12,57].

Antibody titers against RSV above 100 reduce both the frequency and the severity of respiratory tract infections in children; the protection seems to be correlated with antibody titers against RSV. In rats, neutralizing antibody titers above 1:390 reduce by 99% the chance of pulmonary infections, but higher titers (1:3.500) are needed for a similar reduction in nasal symptoms to occur; therefore, the neutralizing antibodies protect better against lower than upper respiratory tract infections[44].

Based on these results, the role played by human immunoglobulin in the protection against RSV has been investigated since the beginning of the 90's. Although safe, the monthly infusion of high doses (750 mg/kg) of normal human immunoglobulin in preterm neonates contains low antibody titers against RSV (only 1:87) and provides no protection against RSV infections[19]. Therefore, a specific immunoglobulin containing high antibody titers against RSV (RSV-IGIV) has been developed.

The specific immunoglobulin (respiratory syncytial virus immunoglobulin – RSV-IGIV, Respigam) contains approximately six times the antibody titers of the normal human immunoglobulin and is 10 times more potent in the reduction of RSV titers after experimental infections in rats. *In vitro* studies demonstrated that RSV-IGIV can neutralize a number of groups A and B RSV strains[3,34,43].

The first multicenter study to demonstrate the effective use of the policlonal immunoglobulin specific against RSV was sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and published by Groothuis et al. (1993).[19] In this study, 249 children under 48 months of age (mean age = 8 months) presenting bronchopulmonary dysplasia due to prematurity (n= 102), congenital heart disease (n= 87) or preterm infants under 35 week-gestational age and below six-month chronological age (n= 60) were evaluated. The children were divided into three groups: A) a group of 81 children received five monthly 750 mg/kg injections of RSV-IGIV immunoglobulin (high dose group = 15 ml/kg); B) a group of 79 children received 150 mg/kg of RSV-IGIV (low dose group = 3 ml/kg) and group C, composed of 89 children who received placebo in five monthly injections. During the follow-up period, 64 episodes of RSV infections were confirmed: 19 in group A (high dose of RSV-IGIV), 16 in group B (low dose of RSV-IGIV) and 29 in group C (control). In group A (high doses of RSV-IGIV) a lower rate of lower respiratory tract infections (7 *versus* 20, compared to the control group, p=0.01), a lower rate of hospital admissions (6 *versus* 18 in the control group), shorter hospital stays (43 *versus* 128 days, p=0.02), shorter stays in intensive care units and lower use of ribavirine (p= 0.05) were observed. In group B (low dose of RSV-IGIV), only a reduction in the number of days of hospitalization in intensive care units was observed, no significant effect on the reduction of lower respiratory tract infections rate being detected.

The adverse effects associated with the use of RSV-IGIV were rare (3%) and mild in most cases. Five children

Table 2 - Major problems associated with the active immunization against RSV.

Problem	Comments
1. Most infections occur in infants	- There are technical and ethical obstacles involved in the vaccination of young infants or pregnant women
2. RSV has a short period of incubation	- The vaccines should stimulate both the serum and the mucosa immunity
3. Glycoproteins F and G are the primary antigens responsible for the immunity	- Glycoproteins are poor immunogenic antigens, particularly in infants
4. There are several strains of RSV	- The vaccines should provide protection against several strains of Types A and B
5. Natural infection does not provide full protection	- Effective vaccines that can provide greater protection than that obtained after the natural infection will hardly be developed.
6. Children who have received the inactivated vaccine presented more severe disease when infected with RSV.	- All the pathophysiologic mechanisms and the immune response to RSV are still unknown.

presented fluid overload associated to RSV-IGIV infusion, but all of them showed a good response to the use of diuretics. Eight children presented a slight reduction in oxygen saturation and six presented fever. Only a more severe reaction was observed – a 17-month old infant developed high-grade fever (T= 40.6ºC) after the second dose of RSV-IGIV, followed by increased respiratory distress. This child presented severe BPD and evoluted to progressive fall in ventilation function, dying three months later. In total, six children died during the follow-up period, five of them presenting congenital heart disease and, in three cases, death was directly associated to the surgical intervention. In none of the cases the death was directly associated to the use of RSV-IGIV.

This study showed that, although the total number of RSV infections has not been significantly reduced in groups 1 and 2, the use of high doses of RSV-IGIV reduced the disease severity, decreasing in up to 63% the rate and duration of hospital stays for RSV, and, in up to 97%, the number of days in ICU[19]. Despite the good results observed in this study, the Food and Drug Administration (FDA) did not approve of the license for RSV-IGIV, due to potential immunoglobulin adverse events and the need for a better study design to evaluate its effectiveness in different groups[57].

In order to better evaluate the safety and effectiveness of high-doses of RSV-IGIV among children with bronchopulmonary dysplasia and/or prematurity status, a new study was conducted (Prevent Study Group) in 54 centers in the United States, in which 510 children with bronchopulmonary dysplasia and/or prematurity status were included and randomly assigned to treatment with high-dose RSV-IGIV (750 mg/kg) or placebo (1% albumin) in five monthly intravenous infusions, during the 1994-1995 RSV season. The children were followed over 30 days after the last infusion. In the group receiving RSV-IGIV, 20 RSV infections (8%) were observed, whereas in the placebo group, 35 (13.5%) were confirmed, with a 41% reduction in the incidence of hospitalizations for RSV in the treated group. For each 100 children treated with RSV-IGIV, a 53% reduction in the number of days of hospitalization was observed in the presence of moderate to severe lower respiratory tract infections and, in the presence of lower respiratory tract infections of any severity, this reduction achieved 38%[43].

The children presented good tolerability to high doses of RSV-IGIV (15 ml/kg). However, in 1.8% of the cases, the infusion had to be discontinued due to volume overload. In total, 8.4% of the children (1% preterm and 13% with BPD) received diuretics in, at least, one infusion, but, in most cases, it was impossible to specify if its use was preventive or therapeutic. Three children included in the RSV-IGIV-treated group presented an increase in the number of CSF cells (20 cells or below); one of them was presumptively diagnosed with enteroviral meningitis (nasopharinx-isolated enterovirus), the other was treated for herpes simplex and presented an improvement and the third one was diagnosed during an investigation for sepsis. Fever was observed in 6% of the treated group and in 2% of the placebo group. One of the most interesting aspects of this study was the finding that the children receiving RSV-IGIV presented a lower incidence of skin rash (38% *versus* 48%) and acute otitis media (27% *versus* 43%), when compared to the placebo group[43].

The role of RSV-IGIV prophylaxis in children with congenital heart disease is controversial. One study conducted by Simões et al. (1998)[50] evaluated the effectiveness and safety of this policlonal immunoglobulin in 416 children with congenital heart disease; 202 received monthly injections of RSV-IGIV and 214 served as a control group. A tendency towards a reduction in the rates and duration of hospital stays was observed among the treated children. However, the children presenting congenital cyanotic heart disease and treated with the specific immunoglobulin presented a greater number of cyanosis episodes and higher rates of complications following cardiac surgery when compared with the control group.

In none of the studies in which the use of the specific immunoglobulin against RSV has been evaluated, it was possible to demonstrate a significant reduction in the mortality rates in the treated groups, when compared to non-treated groups[44], but since the safety and effectiveness of RSV-IGIV in reducing RSV infection complications has been demonstrated, this product was licensed by the USA FDA in January 18, 1996[1,2,34,57].

The use of RSV-IGIV: cost/benefit ratio

The use of RSV-IGIV is approved for children under two years of age who were born with less than 36 weeks of gestation, or who present bronchopulmonary dysplasia. However, for each hospitalization for RSV prevented, it is estimated that 16 preterm infants (12 preterm with BPD or 63 preterm without BPD) should receive prophylaxis[44,46,50].

In the U.S., the cost of five doses of RSV-IGIV ranges from US$3,200 to US$13.988 for infants weighting 1,2 to 10 kg at the beginning of treatment[39,52]. It is estimated that US$ 1,4 billion is necessary to administer RSV-IGIV to all preterm infants. Due to its high cost, it is believed that the use of RSV-IGIV should be reserved only to high-risk groups, such as those with BPD requiring oxygen supplementation or neonates under 32 weeks of gestation, specially those born close to RSV season[44]. A recent study demonstrated that over the two years that followed the beginning of RSV-IGIV use for preterm infants under 32 weeks of ges-

tation, a significant reduction in the disease-related costs (>60%) has been observed, when compared to the two years that preceded the licensing of this immune biologic product[3].

Besides the high cost, the use of RSV-IGIV carries the following problems: it requires the use of a venous access route, hospitalization for several hours for drug infusion, difficulties regarding product availability (blood derivative), potential for severe adverse events (volume overload, aseptic meningitis, anaphylactic reactions in people with IgA deficiency and cyanosis episodes) and possible interference with the immunogenicity of measles, mumps, rubella and varicella live vaccines, which should be postponed for 9 to 10 months[1,13,34,52]. For these reasons, other forms of infection prevention have been investigated, such as the use of monoclonal antibodies[11,13,20,28-30] or new vaccines[4,5,8,13-15,20,23,28,40-42,44,59].

Passive immunization against RSV with monoclonal antibodies

Since the nasal mucosa is the major route for the RSV, the topical use of IgA-type antibodies was thought to protect against the infection. In monkeys, the topical use of murine IgA monoclonal antibodies targeting RSV protein F reduced the RSV replication, but in children, a placebo-controlled study did not demonstrate a good effectiveness of these antibodies, despite the small size of the study[34].

In experimental animals, the parenteral use of monoclonal antibodies can lower in up to 99% the rate of RSV infections. Two large studies have been carried out with monoclonal antibodies, targeting RSV glycoprotein F, given to children through the parenteral route. In a first study, a monoclonal antibody, produced by Smith Kline Beecham (SB 209763) was used in 791 children from 93 centers. The children were randomly assigned to receive monthly injections of 10 mg/kg of the drug or placebo. The results of this study were disappointing, probably due to the low dose or low antiviral activity of the antibodies[34].

In a second study, the parenteral use of humanized monoclonal antibodies produced by MedImmune (MEDI-493, palivizumab, Synagis) showed good results in the prevention of RSV severe infections[26].

Palivizumab contains monoclonal antibodies that bind to RSV surface protein F and exert high activity against group A and B virus; it is produced by genetic engineering techniques (fusion of murine myeloma cell with T lymphocytes in BALB/c rats immunized with the RSV human A2 strain). In order to reduce the potential sensibilization that murine antibodies could cause in humans, the nucleotide sequence that codes for the antibody binding site contains 95% of the aminoacid sequence found in human antibodies and only 5% of that found in murines. This is obtained through the insertion of the gene that codes for a human IgG1 subclass molecule in the genoma of the cell line, which produces the monoclonal antibodies. The murine aminoacid sequence is restricted almost exclusively to the combination sites of the antibody with the virus, which decreases the chances for an individual to present hypersensitivity reactions to the product, due to the production of anti-idiotypical antibodies[13,26].

The Impact-RSV Study Group was the first large study conducted to determine the safety and effectiveness of palivizumab[7]. This study was carried out in 1996-1997 in 139 centers from the U.S. (n=119), Canada (n=9) and the United Kingdom (n=11) and included 1,502 preterm infants (<35 weeks and <6 months of age) or children under two years of age with bronchopulmonary dysplasia requiring continuous medical treatment (i.e., oxygen supplementation, steroids, bronchodilators or diuretics within the last six months). The children were randomly assigned to receive monoclonal antibody (15 mg/kg) or matched placebo intramuscularly in the form of five monthly injections and were followed for 150 days (up to 30 days after the last injection), being evaluated the hospitalization rate, the number of days of hospitalization, the need for oxygen supplementation, the disease severity, the number of days of hospitalization in intensive care unit and the need of mechanical ventilation. More than 93% of the children completed the protocol. The use of palivizumab reduced by 55% the hospitalizations for RSV (10.6% in the placebo group *versus* 4.8% in the monoclonal antibody-treated group; p=0.00004); preterm infants without BPD presented a 78% reduction in the rate of hospitalizations (8.1% *versus* 1.8%; p=0,001) and children with BPD presented a 39% reduction (12.8% *versus* 7.9%; p=0.038). For each group of 100 children followed, it was observed that the treatment with palivizumab reduced significantly the number of days of hospitalization (62,6 days in the placebo group *versus* 36,4 in the palivizumab group), the number of days with increased need of oxygen supplementation (50,6 days in the placebo group *versus* 30,3 days in the palivizumab group) and the number of days with more severe pictures (severity score 3: 47,7 days in the placebo group *versus* 29,6 days in the palivizumab group). The drug was well tolerated with few adverse events occurring at a frequency not significantly superior to that observed in the placebo group. Local reactions occurred, such as mild erythema (1.8% in the placebo group *versus* 2.7% in the treated group), being observed a slight increase in hepatic enzymes in both groups (AST: 1.6% in the placebo group *versus* 3.6% for palivizumab; ALT: 2.0% in the placebo group *versus* 2.3% for palivizumab). Only 0.3% of the children treated discontinued therapy[26].

Comparing the results of this study with the results obtained with the use of the specific immunoglobulin (RSV-IGIV), it was observed that the use of

monoclonal antibodies is safer and more convenient for the child[13]. Whereas the use of high doses of RSV-IGIV requires venous access and several hours of infusion (15 ml/kg dose), the use of monoclonal antibodies is easier through the intramuscular route. In addition, monoclonal antibodies seem to be more effective in the reduction of hospitalization rates in children infected with RSV. The reduction of hospitalization rates obtained with palivizumab and RSV-IGIV were, respectively, 55% (95% CI: 38%-72%) and 41% (95% CI: 10%-72%)[26].

The size of the Impact trial allowed for the evaluation of the efficacy of monoclonal antibodies in different groups, stratified according to the birth weight, gestational age and the presence of BPD. Significant reductions in hospitalizations were observed in the following groups: children over 5 kg (51%, p=0.014) and children < 5 kg (57%, p=0.001); children under 32-weeks (47%; p=0.03) and 32-to-35 week gestational age (80%, p=0.002). The results were similar in all study centers (USA, Canada and United Kingdom)[26].

Palivizumab (Synagis) was licensed in the United States in June 1998 and has been available in several countries since 1999[51]. In Brazil, it is commercialized by Abbott and distributed in packages containing one 100 mg vial of lyophilized powder, which should be stored at 2ºC to 8ºC (do not freeze). According to the manufacturer, palivzumab should be gently reconstituted before use with sterile water and homogenized with slow movements (do not shake the vial). After reconstitution, it should be allowed to rest at room temperature for at least 20 minutes, until the solution becomes clear. It should be used within six hours.

Despite the proven efficacy of palivizumab in 32 to 35 week-gestation children without bronchopulmonary disease (82% reduction in hospitalization rates among 335 children born between the 32nd and the 35th gestational weeks without chronic pulmonary disease)[26], it should be considered that RSV infections rarely are fatal, even in high-risk groups, if the children receive proper medical care, and that the cost of the drug is still too high to be recommended for all preterm infants under 35-week gestation.

It is estimated that 288,000 babies under 36-week gestation and approximately 80,000 under 32-week gestation are born each year in the United States alone[34,44]. Since there are no studies on the cost/benefit ratio of palivizumab, the cost of the drug is high and few experiments exist on this drug, the AAP does not recommend it for all children under 35-week gestation. In the United States, the cost of a 100 mg vial of palivizumab is US$ 900; the cost of the therapy involving five doses of the product for a child under 3 kg weight ranges from US$ 2,250 to US$4,500, depending on whether the product can be or not shared with other clients[13,34,44,51,57]. It is estimated that 17,2 patients need to be treated with the product to prevent hospitalization and that the cost of each hospitalization prevented would achieve approximately US$77,000. This cost is 10 times greater than the cost of hospitalization for non-treated individuals[44].

Therefore, it is suggested that the prophylactic use of monoclonal antibodies, as well as RSV-IGIV, be reserved for higher risk groups and that, for 32 to 35-week gestation children, these drugs are indicated only if the child presents concomitant problems that also predispose for respiratory complications such as, neurologic disturbance, promiscuity (a large number of people sharing the same bedroom, many siblings, nursery or day-care center attendance), domestic exposure to cigarette smoke or a scheduled cardiac surgery. In addition, the availability and access to hospital centers for the treatment of severe respiratory diseases should be taken into consideration. Despite these recommendations, it is essential to remember that most children presenting such prerequisites will not have severe RSV disease and that the costs and logistic difficulties to treat these children can neutralize the possible benefits[1,3,13,44,51,57].

Since 1997, the American Academy of Pediatrics has recommended the use of RSV-IGIV for children under two years of age with previous history of chronic bronchopulmonary disease, who required oxygen supplementation within the previous six months from RSV season[1]. After palivizumab registration, the use of monoclonal antibodies (palivizumab) has been preferred.

The advantages of the use of monoclonal antibodies are summarized in table 3.

Table 3 - Advantages and disadvantages of the use of monoclonal antibodies when compared to the specific immunoglobulin.

Characteristics	Monoclonal antibodies	Specific immunoglobulin
Antibody titers against RSV	Very high	10 to 50 times lower when compared to monoclonal antibodies
Safety	Comparable to placebo	Risk of volume overload
Administration	IM, more convenient and rapid	IV, slow and more expensive
Interference with live virus vaccines	None	Possible. It is recommended to wait up to 9 months to administer live virus vaccines
Cost	High (10% lower than that of RSV-IGIV)	High

Considering that the use of monoclonal antibodies is safer and more convenient for the child than the RSV-IGIV, its use should be considered in the following cases[34]:

1. Children under 24 months of age with chronic pulmonary disease, who required treatment in the last six months;
2. Infants born between the 28th and the 32nd week of gestation, without chronic pulmonary disease, and under six months of age at the beginning of winter;
3. Infants born with less than 28 weeks of gestation, without chronic pulmonary disease and under one year of age at the beginning of RSV season;
4. Infants born with 32 to 35 weeks of gestation without chronic pulmonary disease, who are under six months of age at the beginning of RSV season presenting concomitant risk factors (crowded environments, siblings at school age, domestic exposure to cigarette smoke, multiple parities).
5. Prophylaxis with palivizumab is not recommended for children with congenital heart disease, unless the child is included in the groups mentioned above, and if it presents hemodynamically compensated lesions, such as patent ductus arteriosus or small septal defects.
6. Neither the specific immunoglobulin (RSV-IGIV) nor the monoclonal antibodies (palivizumab) are indicated for the treatment of RSV infections.

Despite the advantages of palivizumab, there is, up to now, no study comparing directly the risks, benefits and costs derived from the use of monoclonal antibodies with specific immunoglobulin. In addition, since RSV-IGIV provides protection against other viral respiratory diseases, in some situations its use can be preferred in certain groups of risk, such as children who have already been receiving replacement therapy with intravenous immunoglobulin for the treatment of immune deficiency, including HIV infection[1,13,34,51].

Passive immunoprophylaxis in immune deficient individuals

The safety and effectiveness of passive immune prophylaxis against RSV is being clinically evaluated in individuals with cellular immune deficiency, particularly those undergoing organ transplants or presenting more severe illnesses. HIV-infected children, although excreting RSV for prolonged periods, do not seem to present more severe illnesses and the routine use of passive prophylaxis with specific immunoglobulin, or with palivizumab, is not recommended. The indication for prophylaxis against RSV has been evaluated in children with cystic fibrosis and asthma, but, up to now, the risks and benefits derived from the use of passive immune prophylaxis are not clear in such situations[34].

New perspectives for the active immunization against RSV

Besides specific immunoglobulin and monoclonal antibodies for nasal and intramuscular use, several new vaccines containing RSV-attenuated strains (cold adapted, temperature sensitive, developed through recombinant technology), vaccines containing purified proteins (developed through recombinant genetic engineering or live-vector produced), synthetic peptide vaccines and DNA vaccines are currently under investigation[4,5,8,13-15,17,18,20,23,28,32,40-42,54,59,60].

Attenuated live virus vaccines – In the 70's, Wright et al. (1976)[59] tested a temperature-sensitive (ts) intranasal vaccine containing attenuated RSV in twenty-five children aged 11 to 19 months, in a randomized and controlled trial (controlled, double-blind protocol). From the 25 children receiving this vaccine (100 $TCID_{50}$), eight were infected with the vaccinal strain, as confirmed by viral shedding or higher serum antibodies; however, the rate of infection with the wild virus was comparable to the groups of children receiving the nasal vaccine or placebo. Despite having failed to provide protection to vaccinated children, unlike the inactivated vaccine, this nasal, live, attenuated virus vaccine showed to be safe, causing no increase in the disease severity in the vaccinated children exposed to and infected with the wild virus in the following year. Therefore, the attenuated virus vaccine becomes the standard for the development of new experimental vaccines[59]. One of the greatest difficulties in the development of vaccines against RSV is to obtain strains properly balanced in terms of immunogenicity, stability and safety. Some attenuated vaccines have shown a low level of immunogenicity while others have shown to be very unstable [8,31].

Since the temperature-sensitive attenuated virus vaccines are not adequately immunogenic, new vaccines have been recently tested in which the virus attenuation is obtained through other techniques, such as expression of interleukins and are presently undergoing clinical trials in animals[5].

Vaccines containing protein F purified

Protein F is the major antigen responsible for the production of antibodies against RSV subtypes A and B; therefore, vaccines containing the purified protein F, obtained through genetic engineering techniques, seem to be highly promising in the prevention of diseases caused by this agent. Groothuis et al. (1998)[20] administered one dose of the PFP-2 vaccine against RSV to 10 children with bronchopulmonary dysplasia and the influenza vaccine to 9 children; 90% (9/10) of the children who received the PFP-2 vaccine presented a (four-fold) increase in serum antibody titers against RSV *versus* 0/11

in those receiving the influenza vaccine. After one year, it was observed that both groups presented similar antibody titers against protein F and neutralizing antibodies against RSV. However, whereas in the control group half of the children (6/11) were hospitalized for RSV respiratory disease, in the vaccinated group, the hospitalization rate for the same reason was only 10% (1/10; p=0.06). The PFP-2 vaccine showed to be safe and immunogenic in children with BPD[20].

Piedra et al. (1996)[41] administered the PFP-2 vaccine against RSV to 17 children with cystic fibrosis (median age=4,5 years) and other 17 children (median age= 5,8 years) received placebo. While the vaccine has failed to fully protect against RSV, a significant reduction (P=0.01) in the number of lower respiratory tract infection episodes was observed in the vaccinated group (0,8) when compared to the control group (2,1), as well as a significant reduction in the days of disease duration (30,5 *versus* 67) in the group receiving the PFP-2 vaccine. The local and systemic reactions to this vaccine were mild in nature, being observed no increase in the disease severity in the reinfected children.

In the following year, the same group of investigators revaccinated the children with the PFP-2 vaccine and demonstrated that the revaccination was quite safe, causing no increase in the adverse events[42].

CONCLUSIONS

Preliminary studies using several vaccines against RSV, both in experimental animals and humans, seem to provide promising results[9,11,19,17]. However, it should be stressed that natural infection does not fully protect against infection and reinfection and, since the immunological factors related to RSV protection have not been fully clarified, it is unlike that an effective vaccine that can provide a high level of protection against this agent can be developed on a short-term basis. Theoretically, the vaccination of pregnant women could benefit neonates. However, the vaccine safety issues in this group make it difficult to conduct studies on this new perspective for the prevention of severe RSV diseases[18,34].

Concluding, it is worth remembering that the most effective way to control nosocomial RSV infections is through careful hand washing, in order to prevent adults (health professionals and relatives) from transmitting the RSV to high-risk children. Thorough hand washing, combined with the use of disposable aprons, gloves, goggles that protect both eyes and nose, can reduce by up to eight times the chances of nosocomial infection[44,47].

RESUMO RHCFAP/3041

BRICKS LF – Prevenção das infecções pelo vírus sincicial respiratório. **Rev. Hosp. Clín. Fac. Med. S. Paulo 56**(3):79-90, 2001.

Em todo o mundo, o vírus sincicial respiratório é o principal agente de infecções agudas das vias aéreas baixas em lactentes jovens e crianças. Aos dois anos de idade, praticamente todas as crianças já foram infectadas, e as reinfeções são comuns, durante toda a vida. Embora a maioria das infecções seja leve, o vírus sincicial respiratório pode causar doenças graves, especialmente em prematuros com displasia broncopulmonar e, nos últimos anos, tem sido identificado como causa importante de infeções respiratórias em pessoas que apresentam comprometimento da imunidade ou outros problemas médicos e em idosos hospitalizados. O impacto econômico dessas infecções faz com que o desenvolvimento de vacinas contra o vírus sincicial respiratório seja altamente desejável, entretanto, o insucesso da primeira vacina inativada contra esse agente dificultou os progressos nesse campo e, até o presente, não há nenhuma vacina licenciada contra o vírus sincicial respiratório. Nos últimos anos, entretanto, o melhor entendimento sobre imunologia e os mecanismos imunopatológicos envolvidos na resposta ao vírus sincicial respiratório propiciaram o desenvolvimento de novas estratégias para a profilaxia ativa e passiva contra essas infecções. Neste artigo, a autora apresenta uma revisão sobre os mais recentes avanços na prevenção das infecções pelo vírus sincicial respiratório, tais como: uso de imunoglobulina humana policlonal, anticorpos monoclonais humanizados (ambos já licenciados para uso em prematuros e crianças com displasia broncopulmonar) e o desenvolvimento de diferentes vacinas que são potenciais candidatas para imunização ativa contra o vírus sincicial respiratório.

DESCRITORES: **Vírus sincicial respiratório. Anticorpos monoclonais. Imunoglobulina. Vacinas. Imunização.**

REFERENCES

1. AMERICAN Academy of Pediatrics - Respiratory syncytial virus. In: PETER G ed. **Report of the Committee on Infectious Disease**, 24th ed, Chicago, Elk Grove Village, 1997. p. 443-448.

2. AMERICAN Academy of Pediatrics Committee on Infectious Disease, Committee on Fetus and Newborn - Respiratory syncytial virus immune globulin intravenously: Indications for use. **Pediatrics** 1997;**99**:645-650.

3. ATKINS JT, KARIMI P, MORRIS BH et al. - Prophylaxis for respiratory syncytial virus with respiratory syncytial virus-immunoglobulin intravenous among preterm infants of thirty-two weeks gestation and less: reduction in incidence, severity of illness and cost. **Pediatr Infect Dis J** 2000, **19**:138-43.

4. BELSHE RB, ANDERSON EL & WALSH EE - Immunogenicity of purified F glicoprotein of respiratory syncytial virus: clinical and immune responses to subsequent natural infection in children**. J Infect Dis** 1993;**168**:1024-1029.

5. BUKREYEV A, WHITEHEAD SS, PRUSSIN C et al. - Effect of coexpression of interleukin-2 by recombinant respiratory syncytial virus on virus replication, immunogenicity, and production of other cytokines. **J Virol** 2000;**74**, 7151-7157.

6. CHANOCK RM, PARROT RH, CONNORS et al. - Serious respiratory tract disease caused by respiratory syncytial virus. Prospects for improved therapy and effective immunization. **Pediatrics** 1992;**90**:137-143.

7. COX MJ, AZEVEDO RS, CANE PA et al. Seroepidemiological study of respiratory syncytial virus in São Paulo State, Brazil. **J Med Virol** 1998;**55**:234-239.

8. CROWE JE Jr - Immune responses of infants to infection with respiratory viruses and live attenuated respiratory virus candidate vaccines. **Vaccine** 1998;**12**:783-790.

9. CUNNINGHAM CK, MCMILLAN JA & GROSS SJ - Rehospitalization for respiratory illness in infants of less than 32 weeks gestation. **Pediatrics** 1991;**88**:527-32.

10. DINIZ EMA, VIEIRA RA, ISHIDA MA et al. - Clinical and laboratorial evaluation of neonates with respiratory syncytial virus interstitial pneumonia. In: COSMI EV - INTERNATIONAL CONGRESS ON NEW TECHNOLOGIES IN REPRODUCTIVE MEDICINE, NEONATOLOGY AND GYNECOLOGY. 2nd. Porto Conte, Itália. Monduzzi Ed., 1999. (18-23 de Setembro). p.531-535.

11. DUDAS RA & KARRON RA - Respiratory syncytial virus vaccines. **Clin Microbiol Rev** 1998;**11**:430-439.

12. DURIGON EL, TAKAHASHI VNVO, SOARES et al. - Vírus respiratório sincicial humano. Revisão e levantamento dos dados brasileiros. Universidade de São Paulo. Departamento de Microbiologia – Instituto de Ciências Biomédicas, 2000. p. 1-16. (Monografia distribuída pela Abbot – Divisão Hospitalar).

13. ENGLUND JA - Prevention strategies for respiratory syncytial virus: passive and active immunization. **J Pediatr** 1999;**135**:S38-S44.

14. FALSEY AR & WALSH EE - Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in ambulatory adults over age 60. **Vaccine** 1996;**14**:1214-1218.

15. FALSEY AR & WALSH EE - Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in the institutionalized elderly. **Vaccine** 1997;**15**:1130-1132.

16. GLEZEN WP, PAREDES A, ALLISON JE et al. - Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethic group, and maternal antibody level. **J Pediatr** 1981;**98**;708-715.

17. GONZALEZ IM, KARRON RA, EICHELBERGER M et al. Evaluation of the live attenuated cpts 248/404 RSV vaccine in combination with a subunit RSV vaccine (PFP-2) in healthy young and older adults. **Vaccine** 2000;**18**:1763-72.

18. GRAHAM BS - Pathogenesis of respiratory syncytial virus vaccine-augmented pathology. **Am J Respir Crit Care Med** 1995;**152**:S63-66.

19. GROOTHUIS JR, SIMOES EAF, LEVIN MJ et al. Prophylatic administration of respiratory syncytial virus immunoglobulin to high-risk infants and young children. **N Engl J Med** 1993;**329**:1524-1530.

20. GROOTHUIS JR, KING SJ, HOGERMAN DA et al. Safety and immunogenicity of a purified F protein respiratory syncytial virus (PFP-2) vaccine in seropositive children with bronchopulmonary dysplasia. **J Infect Dis** 1998;**177**;467-469.

21. GROSS M, BRUNE T, JORCH G et al. Significance of respiratory syncytial virus (RSV) infection in the 1st year of life. **Infection** 2000;**28**:34-37.

22. HALL C & MCCARTHY CA - Respiratory syncytial virus. In: MANDELL GL, DOUGLAS RG Jr & BENNETT JE eds. - **Principles and Practice of Infectious Diseases.** 4th ed, New York, NY. Churchill Livingston, 1995:1501-1519.

23. HALL CB - Respiratory syncytial virus: a continuing culprit and conudrum. **J Pediatr** 1999;**135**:S2-S7.

24. HAY JW, ERNST RL & MEISSNER HC - Respiratory syncytial virus immune globulin: a cost-effectiveness analysis. **Am J Managed Care** 1996;**2**:841-56.

25. IMAZ MS, SEQUEIRA MD, VIDELA C et al. - Clinical and epidemiologic characteristics of respiratory syncytial virus subgroups A and B infections in Santa Fe, Argentina. **J Med Virol** 2000; **61**:76-80.

26. IMPACT-RSV study group - Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. **Pediatrics** 1998;**102**:531-537.

27. IZURIETA HS, THOMPSON, WW, KRAMARZ et al. - Influenza and the rates of hospitalization for respiratory disease among infants and young Children. **N Engl J Med** 2000;**342**:232-239.

28. KARRON RA & AMBROSINO DM - Respiratory syncytial virus vaccines. **Pediatr Infect Dis J** 1998,**17**:919-20.

29. LANGLEY JM, LEBLANC JC, WANG EEL et al. – Nosocomial respiratory syncytial virus infection in Canadian pediatric hospital: a pediatric investigators collaborative network in Canada. **Pediatrics** 1997;**100**:943-946.

30. LA VIA WV, MARKS MI & STUTMAN HR - Respiratory syncytial virus puzzle: clinical features, pathophysiology, treatment, and prevention. **J Pediatr** 1992;**121**:503-510.

31. LOVEYS DA, KULKARNI S & ATREYA PL - Role of type I IFNs in the vitro attenuation of live, temperature-sensitive vaccine strains of human respiratory syncytial virus. **Virology** 2000; **271**:390-400.

32. MALHOTRA A, KRILOV LR - Influenza and respiratory syncytial virus. **Pediatr Clin North Am** 2000;**47**:353-72.

33. MCBRIDE JT - Pulmonary function changes in children after respiratory syncytial virus infection in infancy. **J Pediatr** 1999;**135**:S28-S32.

34. MEISSNER HC, WELLIVER RC, CHARTRAND AS et al. - Immunoprophylaxis with palivizumab, a humanized respiratory syncytial virus monoclonal antibody, for prevention of respiratory syncytial virus infection in high risk infants: a consensus opinion. **Pediatr Infect Dis J** 1999;**18**:223-31.

35. MIYAO CR, GILIO AE, VIEIRA S et al. - Infecções virais em crianças internadas por doença aguda do trato respiratório inferior. **J Pediatr** (Rio J.) 1999;**75**:334-344.

36. NASCIMENTO JP, SIQUEIRA MM, SUTMOLLER F et al. – Longitudinal study of acute respiratory diseases in Rio de Janeiro. Occurence of respiratory viruses during four consecutive years. **Rev Inst Med Trop São Paulo** 1991;**33**:287-296.

37. NAVAS L, WANG E, DE CARVALHO V et al. - Improved outcome or respiratory syncytial virus infection in high-risk hospitalized population of Canadian children. **J Pediatr** 1992;**121**:348-354.

38. NOWAK-WEGRZYN A & LEDERMAN HM - Supply, use, and abuse of intravenous immunoglobulin. **Curr Opin Pediatr** 1999;**11**:533-539.

39. O'SHEA TM, SEVICK MA & GIVNER LB - Costs and benefits of respiratory syncytial virus immunoglobulin to prevent hospitalization for lower respiratory tract illness in very low birth weight infants. **Pediatr Infect Dis J** 1998;**17**:587-93.

40. PARADISO PR, HILDRETH SW, HOGERMAN DA et al. - Safety and immunogenicity of a subunit respiratory syncytial virus vaccine in children 24 to 48 months old. **Pediatr Infect Dis J** 1994;**13**:792-798.

41. PIEDRA PA, GRACE S, JEWELL A et al. - Purified fusion protein vaccine protects against lower respiratory tract illness during respiratory syncytial virus season in children with cystic fibrosis. **Pediatr Infect Dis J** 1996;**15**:23-31.

42. PIEDRA PA, GRACE S, JEWELL A et al. - Sequential annual administration of purified fusion protein vaccine against respiratory syncytial virus in children with cystic fibrosis. **Pediatr Infect Dis J** 1998;**17**:217-224.

43. PREVENT Study Group. - Reduction of respiratory syncytial virus hospitalization among premature infants and infants with bronchopulmonay displasia using respiratory syncytial virus immunoglobulin prophylaxis. **Pediatrics** 1997;**99**:93-99.

44. PROBER CG & SULLENDER WM - Advances in prevention of respiratory syncytial virus infections. **J Pediatr** 1999; **135**:546-58.

45. RAZA MW, BLACKWELL CC, ELTON RA et al. - Bactericidal activity of a monocytic cell line (THP-1) against common respiratory tract bacterial pathogens is depressed after infection with respiratory syncytial virus. **J Med Microbiol** 2000,**49**:227-233.

46. ROBBINS JM, TILFORD JM, JACOBS RF et al. - A number-needed-to-treat analysis of the use of respiratory syncytial virus immune globulin to prevent hospitalization. **Arch Pediatr Adolesc Med** 1998;**152**:358-366.

47. RODRIGUEZ WJ - Management strategies for respiratory syncytial virus infections in infants. **J Pediatr** 1999; **135**:S45-S50.

48. SELWYN BJ - On behalf of the coordinated data group of bostid researchers. The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. **Rev Infect Dis** 1990; **12** (suppl 8):S870-88.

49. SHAY DK, HOLMAN, RC, NEWMAN RD et al. - Bronchiolitis-associated hospitalizations among US children, 1980-1996. **JAMA** 1999;**282**:1440-1446.

50. SIMOES EAF, SONDHEIMER HM, TOP H et al. - Respiratory syncytial virus immune globulin for prophylaxis against respiratory syncytial virus disease in infants and children with congenital heart disease. **J Pediatr** 1998;**133**:492-9.

51. SIMOES EA. Respiratory syncytial virus infection. **Lancet** 1999;**354**:847-52.

52. TAKUR BK, WU LR & SCHAEUFELE JF - RSV-IGIV Therapy: a cost/benefit analysis. **Pediatrics** 1997;**100**;3:417.

53. TREANOR JJ, MATTISON HR, DUMYATI G et al. - Protective efficacy of combined live intranasal and inactivated influenza A virus vaccines in the elderly. **Ann Intern Med** 1992;**117**:625-633.

54. TRISTAM DA, WELLIVER RC, MOHAR CK et al. - Immunogenicity and safety of respiratory syncytial virus subunit vaccine in seropositive children 18-36 months old. **J Infect Dis** 1994;**167**:191-5.

55. WANG EEL, LAW BJ, STEPHENS D et al. - Pediatric Investigator Collaborative Network on Infections in Canada prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory infection**. J Pediatr** 1995;**126**:212-219.

56. WALSH EE, MCCONNOCHIE KM, LONG CE et al. - Severity of respiratory syncytial virus is related to virus strain. **J Infect Dis** 1997;**175**:814-820.

57. WELLIVER RC - Respiratory syncytial virus immunoglobulin and monoclonal antibodies in the prevention and treatment of respiratory syncytial virus infection. **Semin Perinatol** 1998;**22**:87-95.

58. WILSON SD, ROBERTS K, HAMMOND K et al. - Estimation of incidence of respiratory syncytial virus infections in schoolchildren using salivary antibodies. **J Med Virol** 2000;**61**:81-84.

59. WRIGHT PF, SHINOZAKI T, FLEET W, et al. - Evaluation of a live, attenuated respiratory syncytial virus vaccine in infants. **J Pediatr** 1976;**88**:931-936.

60. WRIGHT PF, KARRON RA, CROWE JE Jr et al. - Evaluation of a live, attenuated respiratory syncytial virus (RSV) vaccine candidate, cpts 248/1404, in infancy [abstract] **Pediatr Res** 1998;**43**:161 A.

61. YÜKSEL B & GRENOUGH A - Birth weight and hospital readmission of infants born prematurely. **Arch Pediatr Adolesc Med** 1994;**148:** 384-388.

62. ZERR DM & FRENKEL LM - Advances in antiviral therapy. **Curr Opin Pediatr** 1999;**11**:21-27.

Received for publication on February 14, 2001.