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Human respiratory syncytial virus and other viral infections in infants receiving palivizumab

Guy Boivin^{a,b,*}, Georges Caouette^{b,c}, Line Frenette^{b,c}, Julie Carbonneau^a,
Manale Ouakki^d, Gaston De Serres^{b,d}

^a Research Center in Infectious Diseases, CHUQ-CHUL, Québec City, QC, Canada

^b Laval University, Québec City, QC, Canada

^c Sainte-Foy Pediatric Clinic, Québec City, Canada

^d Public Health Research Unit, Québec City, QC, Canada

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Abstract

Background: Palivizumab is a humanized monoclonal antibody that prevents severe human respiratory syncytial virus (HRSV) infections. **Objectives:** We determined the etiology of respiratory viral infections in palivizumab recipients, and monitored the clinical outcome and HRSV genotype in HRSV-infected infants. **Study design:** Nasopharyngeal aspirates (NPAs) were collected from children receiving palivizumab who consulted or were hospitalized for acute respiratory tract infection (ARTI) during the 2004–2005 season. Viral cultures and multiplex RT-PCR for influenza A/B, HRSV and human metapneumovirus were performed. The fusion (F) gene of HRSV amplicons was also sequenced. **Results:** Among 116 enrolled patients, 51 (44%) had ≥ 1 episode of ARTI for a total of 93 visits. At least one virus was identified in 33 (36%) of the 93 NPA samples; HRSV accounted for 11 (33%) of confirmed viral etiologies. Compared to subjects who had other viral ARTI, HRSV-positive subjects had less fever ($p=0.01$) and tended to have more bronchiolitis ($p=0.07$). Ten subjects (11 visits) developed HRSV infection, although only one was hospitalized. HRSV was detected after a median of 5.5 palivizumab doses and a median of 14 days after the last dose. One of the 11 HRSV strains tested had a F mutation located in the palivizumab-binding site. **Conclusion:** HRSV is still a major cause of ARTI in children receiving palivizumab, although the outcome of infected children appears mild. © 2007 Elsevier B.V. All rights reserved.

Keywords: Human respiratory syncytial virus; Palivizumab; Resistance

1. Introduction

Human respiratory syncytial virus (HRSV) is a major cause of lower respiratory tract infection in premature babies, infants less than 6 months old and elderly institutionalized subjects (Greenough, 2002; Welliver, 2003). Although, only 1% of HRSV-infected infants require hospitalization, such infections are associated with 50–90% of hospitalizations attributable to bronchiolitis, and 20–50% of hospitalizations attributable to pneumonia (Ogra, 2004). In the US, it has

been estimated that HRSV infections account for approximately 90,000 hospitalizations and 4000–17,000 deaths yearly (Ogra, 2004; Thompson et al., 2003).

Palivizumab (Synagis, MedImmune Inc., Gaithersburg, MD) is a humanized monoclonal antibody directed against the HRSV fusion (F) glycoprotein (Johnson et al., 1997; Meissner, 2003). Palivizumab is recommended during the HRSV season for premature babies (≤ 32 weeks of gestation and a subset of those between 32 and 35 weeks) and infants < 2 years of age with chronic lung problems or congenital heart disease (Feltes et al., 2003; The Impact-RSV Study Group, 1998).

Despite the increased use of palivizumab, there is a paucity of data regarding the impact of such immunoprophylaxis on the viral etiology of acute respiratory tract infections (ARTIs)

* Corresponding author at: CHUQ (CHUL), Room RC-709, 2705 Blvd Laurier, Sainte-Foy, Quebec, Canada G1V 4G2. Tel.: +1 418 654 2705; fax: +1 418 654 2715.

E-mail address: Guy.Boivin@crchul.ulaval.ca (G. Boivin).

Table 1
Demographic and clinical characteristics of 116 infants receiving palivizumab in 2004–2005

	Mean	Median	Range (min–max)	%
Age at birth (weeks)	32.2	32.3	16.4 (24.6–41.0)	
Weight at birth (g)	1919.8	1814.0	3544 (567–4111)	
Age at first palivizumab dose (months)	5.1	3.52	35.16 (0.23–35.39)	
No. of doses of palivizumab received	5.7	6	7 (1–8)	
Prematurity <33 weeks				64.7
Prematurity 33–35 weeks + risk factors				14.7
Gender = male				58.6
≥ 1 brother or sister in household				64.6
Kindergarten frequentation				4.3
Smokers in household				20.7
Breastfeeding				39.6
Underlying diseases				
Pulmonary				24.1
Cardiac				7.8
Metabolic/genetic				6.9
Neurologic				0
Previous respiratory infections				
Bronchiolitis				12.1
Pneumonitis				8.6
Otitis media				2.6
Usual medication				
Oxygen				4.3
Bronchodilators				13.8
Steroids				6.9
Cardiac drugs				4.3
Influenza vaccination in 2004–2005				31.9
Palivizumab in 2003–2004				8.9

in treated infants. The purpose of this study was 2-fold: to determine viral etiologies of both upper and lower respiratory tract infections in palivizumab recipients; and to monitor the clinical outcome and the viral F protein genotype in HRSV-infected infants.

2. Materials and methods

2.1. Study design

Parents of all infants who received at least 1 dose of palivizumab during the 2004–2005 HRSV season at a large private pediatric clinic in Québec City, QC, Canada were invited to participate. Informed consent was obtained for 90% of eligible participants (a few patients living in remote areas were not enrolled), and demographic and medical questionnaires were completed. Parents were advised to contact a research nurse whenever their child was experiencing a respiratory tract infection (with or without fever) severe enough to warrant medical evaluation. When contacted, the research nurse scheduled a medical appointment on the same day at the clinic (week days) or told the parents to consult at the emergency room of the Québec City pediatric hospital (weekends and holidays). At both sites, physicians were instructed to collect a nasopharyngeal aspirate (NPA) for virological anal-

yses and to fill out a questionnaire on clinical symptoms. In addition, at monthly intervals (at the time of palivizumab administration), parents were asked about consultations or hospitalizations of their child. The study was approved by the ethics committee of the Centre hospitalier universitaire de Québec.

2.2. Virological analyses

An aliquot (500 µl) of each NPA was diluted 1:1 in Hanks medium and inoculated on the day of collection on a panel of ten continuous cell lines for viral cultures as previously described (Boivin et al., 2003). Respiratory viruses typically identified by cell culture in this laboratory include: influenza A and B, parainfluenza (PIV) 1–4, HRSV, human metapneumovirus (HMPV) and adenoviruses. In addition, a second aliquot (200 µl) of the NPA was reserved for a multiplex real-time PCR assay that amplifies influenza A matrix, influenza B matrix, HRSV F protein and HMPV nucleoprotein genes as previously described (Boivin et al., 2003; Hamelin et al., 2005). The lower limit of detection for the multiplex assay was 10/50 copies for influenza A/B, 100 copies for HRSV and 50 copies for HMPV using transcribed plasmids. The entire F gene sequence was determined from amplicons from each HRSV-positive specimen and was aligned with corresponding sequences of the A2 strain (GenBank accession

number M11486) for genotype A viruses or with the B1 strain (GenBank accession number AF013254) for genotype B viruses.

2.3. Statistical analyses

Proportions were compared using the Fisher's exact test. Means were compared using the Kruskal–Wallis test. All tests of statistical significance were two-tailed and significance was set at the 5% level. The SAS Institute software (version 9.1) was used for data statistical analysis.

3. Results

3.1. Demographic and clinical characteristics of the population

A total of 116 infants who received at least 1 dose of palivizumab were recruited in the study (Table 1). The mean age at birth was 32.2 weeks. The indication for palivizumab was prematurity <33 weeks for 75 (64.7%), prematurity between 33 and 35 weeks with risk factors for 17 (14.7%), pulmonary conditions only for 7 (6.0%), cardiac conditions only for 7 (6.0%), and other conditions for 10 (8.6%). Seven premature children also had cardio-pulmonary conditions. A total of 44.0% of the subjects had at least one underlying disease and 4.3% were receiving chronic oxygen therapy at the beginning of the study. The mean age at the first dose of palivizumab was 5.1 months and the mean number of doses of palivizumab received per participant during the 2004–2005 HRSV season was 5.7 (range: 1–8). An interval of ≤ 30 days between palivizumab injections occurred for 84% of 114 patients between the first and second dose; 94.5% of 109 patients between the second and third dose; 91% of 101 patients between the third and fourth dose; 93.5% of 92 patients between the fourth and fifth dose; 96% of 79 patients between the fifth and sixth dose; 98% of 45 patients between the sixth and seventh dose; and 100% of 2 patients between the seventh and eighth dose.

3.2. Consultations and hospitalizations for ARTI

Among the 116 patients enrolled in the study, 51 (44%) consulted for ARTI for a total of 93 visits (78 outpatient consultations and 15 hospitalizations) and a mean of 1.8 visits/subject. Due to three missing questionnaires, 90 visits from 50 subjects were analyzed. The mean/median age at the time of the visit was 7.8/7.4 months and 12.7/7.6 months for outpatients and inpatients, respectively ($p=0.9$). The mean number of days between onset of symptoms and collection of NPA was 5.4 (range: 0–30 days) and the NPA was collected within 7 days of onset of symptoms in 73% of visits (76% of outpatient consultations and 57% of hospitalizations). The most frequent clinical diagnoses by the attending physicians included an upper respiratory tract infection (URTI) for 73%

of visits (78% of outpatient consultations and 50% of hospitalizations); a bronchiolitis (defined clinically) for 40% of visits (42% of outpatient consultations and 29% of hospitalizations); and pneumonitis (defined by new infiltrates on chest X-ray) for 3.3% of visits (1.3% of outpatient consultations and 14% of hospitalizations, $p=0.06$). Other diagnoses reported included otitis media (6.7%), pharyngitis (4.4%), croup (3.3%) and a flu-like illness (3.3%).

3.3. Virological findings

At least one virus was identified by viral culture and/or by multiplex real-time RT-PCR in 33 (35.5%) of the 93 NPA specimens collected. Among the viruses identified, there were 11 HRSVs (10 by RT-PCR and 6 by culture), 8 PIVs (4 PIV-3, 2 PIV-1 and 2 PIV-4 by culture), 5 HMPVs (5 by RT-PCR and 3 by culture), 5 adenoviruses (by culture), 2 influenza A (by both RT-PCR and culture), 1 influenza B (by both RT-PCR and culture) and 1 co-infection of HMPV and adenovirus (by a combination of RT-PCR and culture).

3.4. Characteristics of viral infections

Due to the small number of subjects with confirmed viral etiologies, three groups were considered for analysis: HRSV-positive, other virus-positive and virus-negative patients (Table 2). The mean age at the time of NPA (7.4, 7.6 and 9.2 months for the three groups, respectively; $p=0.97$) and the mean interval between onset of symptoms and collection of NPA (5.5, 3.9 and 5.9 days for the three groups, respectively; $p=0.32$) were similar for the three groups when all visits were considered. There were no significant differences between the groups for the presence of cough ($p=0.10$) and wheezing ($p=0.41$), but HRSV-positive children had significantly less fever ($p=0.01$) than children in the other two groups. Compared to children with other viral infections, HRSV-infected subjects had more bronchiolitis (73% vs 36%, $p=0.07$), but less URTI (36% vs 77%, $p=0.05$). There were only three palivizumab-treated children with confirmed pneumonitis (one was positive for HMPV and two had no virus detected). Among the 14 hospitalized children, one each had HRSV, HMPV, adenovirus and influenza B infections and 10 had no viral etiologies.

3.5. Specific characteristics of HRSV-positive children on palivizumab

A total of 11 NPAs were positive for HRSV from 10 children (subject 002 had two positive NPAs during consecutive visits 5 days apart) (Table 3). Thus, 8.6% (10/116) of all recruited infants on palivizumab, or 20% (10/50) of those who consulted or were hospitalized for ARTI, were positive for HRSV. These HRSV infections occurred throughout the study period with cases from the end of January to the end of April. By comparison, the peak of HRSV activity in the Province of Quebec during the same year extended from

Table 2
Clinical features of subjects on palivizumab with HRSV infections or other viral infections

	HRSV			Other viral infections ^a			No virus detected		
	All visits (n = 11)	Outpatient consultations (n = 10)	Hospitali- zations (n = 1)	All visits (n = 22)	Outpatient consultations (n = 19)	Hospitali- zations (n = 3)	All visits (n = 57)	Outpatient consultations (n = 47)	Hospitali- zations (n = 10)
Mean age at the time of NPA (months)	7.4	7.8	3.6	7.6	7.7	6.5	9.2	7.8	15.6
Mean interval between symptoms onset and NPA (days)	5.5	5.1	9	3.9	3.7	5.3	5.9	5.8	6.3
Fever ^b (%)	9.1	10	0	59.1	57.9	66.7	31.6	25.5	60
Cough (%)	100	100	100	90.9	89.5	100	77.2	80.8	60
Wheezing (%)	45.5	50	0	22.7	21.1	33.3	31.6	36.2	10
URTI ^b (%)	36.4	40	0	77.3	78.9	66.7	78.9	85.1	50
Bronchiolitis (%)	72.7	70	100	36.4	36.8	33.3	35.1	38.3	20
Pneumonitis (%)	0	0	0	4.6	0	33.3	3.5	2.1	10
Croup ^b (%)	9.1	10	0	9.1	10.5	0	0	0	0
Otitis media (%)	9.1	10	0	0	0	0	8.8	6.4	20
Pharyngitis (%)	9.1	10	0	0	0	0	5.3	6.4	0
Flu-like illness ^b (%)	0	0	0	13.6	15.8	0	0	0	0

Note: URTI: upper respiratory tract infection; NPA: nasopharyngeal aspirate; HRSV: human respiratory syncytial virus.

^a Infections with influenza A or B, parainfluenza viruses, human metapneumovirus and adenoviruses.

^b $p < 0.05$ by comparing the three study groups.

the beginning of February until mid-March (data not shown). Seven of the 10 HRSV-positive subjects received palivizumab because of prematurity of <33 weeks of gestation, whereas three were born at 33 weeks of gestation and had other risk factors. The mean age of the ten HRSV-positive subjects at the time of first NPA collection was 7.5 months (median: 7.1, range: 3.6–11.7 months) and these children had received a mean of 5.3 doses of palivizumab (median: 5.5, range: 4–7). The mean interval of time between the last dose of palivizumab and detection of HRSV was 16.3 days (median: 14, range: 6–28 days). Seven (70%) of the 10 HRSV-positive subjects had a diagnosis of bronchiolitis although only one (10%) was hospitalized for a period of 7 days (Table 3). This child developed a bronchiolitis that necessitated supplemental oxygen 10 days after the fourth palivizumab injection.

Genotyping of the 11 HRSV strains from 10 subjects revealed the presence of 6 group B and 5 group A strains. Sequence analysis of the HRSV F protein genes amplified directly from clinical specimens (except for strain 029 amplified from the viral isolate) revealed the presence of a few non-silent mutations (Table 3). Notably, the HRSV B strain from patient 038 contained an amino acid change at codon 272 (K/E), which is a part of the known binding epitope for palivizumab (Zhao et al., 2004a). This child received the highest number of palivizumab doses (7) and had a non-complicated bronchiolitis that did not necessitate hospitalization.

4. Discussion

Approximately 10% (10/116) of children receiving palivizumab prophylaxis in the cohort studied experienced

HRSV infections severe enough for their parents to seek medical attention during the 2004–2005 season. Although, 7 of these HRSV-positive children had a diagnosis of bronchiolitis, most cases were mild and only one child (10%) required hospitalization. HRSV infections occurred after a significant number of doses of palivizumab (median of 5.5 doses) and relatively shortly after a dose (median of 14 days). Most cases of HRSV infection could not be explained by the emergence of resistance to palivizumab with the possible exception of one child.

Palivizumab is a humanized neutralizing immunoglobulin G1 monoclonal antibody directed against the F protein of HRSV that is approved for the prevention of severe HRSV infections in high-risk children (The IMPact-RSV Study Group, 1998). Cotton rat studies showed that mean serum levels of palivizumab of 40 µg/ml resulted in a 2-log (99%) reduction of HRSV titers in lungs (Johnson et al., 1997, 1999). The protective effect of palivizumab was confirmed in a large multicenter clinical trial of 1502 high-risk children who received monthly antibody administration. This study demonstrated a 55% reduction in HRSV-related hospitalization (The IMPact-RSV Study Group, 1998). The protective effect of palivizumab was also confirmed in a subgroup of young children with hemodynamically significant congenital heart disease (Feldes et al., 2003).

We systematically assessed the viral etiologies and clinical outcome associated with both upper and lower respiratory tract infections (severe enough to warrant medical consultation) in 116 high-risk patients who received palivizumab during the 2004–2005 winter season. Approximately 2/3 of these children were born prematurely before 33 weeks of gestation and 44% had an underlying disease. The mean number of palivizumab doses received was 5.7 per child and

Table 3
Characteristics of children with HRSV infections while on palivizumab

Patient	Visit	Age (months)	Date of NPA	# Doses before NPA	Time since last dose (days)	Age at birth (weeks)	Diagnosis	Hospitalization (days)	Cult.	RT-PCR (type)	Amino acid change (F gene)
002	6	6	01-26-2005	4	15	31.6	Bronchiolitis	No	-	+(B)	-
002	7	6	01-31-2005	4	20	31.6	Bronchiolitis	No	-	+(B)	-
010	3	6	02-04-2005	4	24	31.7	Bronchiolitis	No	+	+(A)	-
022	1	6	03-16-2005	6	8	33.1	URTI	No	-	+(A)	410(L/V), 422(C/G), 485(S/F)
029	1	10	04-05-2005	6	28	30.3	Bronchiolitis	No	+	-(A) ^a	-
038	1	7	04-21-2005	7	8	32.7	Bronchiolitis, AOM	No	-	+(B)	272(K/E)
045	3	8	03-11-2005	5	23	25.0	Croup	No	+	+(B)	-
052	1	11	04-13-2005	6	28	26.4	URTI	No	+	+(B)	-
058	4	6	04-19-2005	7	6	32.9	Bronchiolitis	No	+	+(B)	107(A/V)
062	2	3	02-18-2005	4	10	33.4	Bronchiolitis	Yes (7)	+	+(A)	183(N/D)
067	1	7	02-18-2005	4	10	33.7	Bronchiolitis	No	-	+(A)	-

Note: URTI: upper respiratory tract infection; AOM: acute otitis media; NPA: nasopharyngeal aspirate.

^a Genotype and F sequence determined from viral isolate since RT-PCR on clinical sample was negative.

38.8% (45/116) received more than 6 doses, highlighting the extended HRSV period in our community during that study. Despite palivizumab prophylaxis, HRSV was the most frequently detected virus, accounting for 33% of ARTI with confirmed viral etiology. HRSV-infected children had significantly less fever than subjects with other viral infections, but there was no significant difference in the rate of wheezing. Other viruses such as HMPV and rhinoviruses have been associated with wheezing episodes and exacerbation of asthma (Jartti et al., 2002; Williams et al., 2004). Also, HRSV-infected children were more frequently diagnosed with bronchiolitis than subjects with other viral infections ($p=0.07$), but had less frequent episodes of URTI ($p=0.05$). Thus, bronchiolitis was not completely prevented in children receiving palivizumab. However, among the 7 children with confirmed HRSV bronchiolitis, only one (the youngest child) required hospitalization and none had pneumonitis. Overall, such data confirm results from a previous placebo-controlled trial that showed that the main effect of palivizumab was to reduce hospitalization in patients with HRSV infections (The Impact-RSV Study Group, 1998).

An unexpected result of this study was the timing of HRSV infections in our palivizumab-treated children. As shown in Table 3, all HRSV infections occurred after a minimum of 4 doses of palivizumab (median 5.5) and a median of 14 days after the last dose. These breakthrough infections could not be explained by more intense viral circulation in the community since they occurred throughout the study period. Previous studies have shown that mean trough serum antibody concentrations increase with the number of palivizumab doses to reach 72 $\mu\text{g/ml}$ after the fourth dose (The Impact-RSV Study Group, 1998) and that levels of serum neutralizing antibody titers are good correlates of protection against HRSV (Piedra et al., 2003). An explanation for the occurrence of such HRSV infections may be that inadequate antibody levels were present in some children. Alternatively, palivizumab could have lost neutralizing activity due to emergence of specific viral mutations or other factors, such as specific cell-mediated immunity, may be involved in protection against HRSV infections.

In vitro and animal studies for selection of palivizumab-resistant HRSV escape mutants have revealed a critical region encompassing amino acids 262–276 of the F protein (Beeler and van Wyke Coelingh, 1989; Zhao et al., 2004a,b; Zhao and Sullender, 2005). In particular, mutations at codon 272 (Lys \rightarrow Met, Gln) and codon 268 (Asn \rightarrow Ile) were associated with complete and partial in vitro resistance, respectively, to palivizumab. Of note, only the 272 mutants were associated with resistance to infused antibody in cotton rats (Zhao et al., 2004a,b; Zhao and Sullender, 2005). To date, there have been no reports of palivizumab-resistant HRSV strains in children, although only a limited number of viruses from infants receiving prophylaxis have been evaluated (DeVincenzo et al., 2004; Johnson et al., 1997). Of the 6 F protein amino acid changes found in our study, only one (Lys 272 Glu) was located in the putative drug-binding

site and could potentially confer resistance to palivizumab. This mutant virus was selected after 7 doses of palivizumab and did not grow initially in cell culture (only RT-PCR was positive). Furthermore, the infected child had an uneventful bronchiolitis. These facts could suggest that this mutant virus had decreased viral fitness or that the antibody was still partially effective. Additional work is also required to evaluate the potential of a new humanized monoclonal antibody derived from palivizumab (Motavizumab, MedImmune Inc.), with a 70-fold increase in binding affinity, against some palivizumab-resistant strains (Mejias et al., 2005). The relevance of the other HRSV F protein changes detected in our study is unclear. A comparative analysis of our strains with 41 HRSV A and 15 HRSV B F protein sequences available in GenBank showed that only the Ser485Phe mutation had been previously reported in only one other HRSV A isolate (data not shown).

Our study has some limitations. First, we had no control (untreated) group to compare viral etiologies and outcomes with our palivizumab recipients. However, the inclusion of such subjects would have been unethical at the time of this study. Second, only a small percentage (36%) of ARTI had a confirmed viral etiology, which probably reflects the absence of specific RT-PCR assays to detect rhinoviruses, coronaviruses and human bocavirus. Also, we did not perform palivizumab neutralization studies for the HRSV strains detected in our study. Lastly, not all eligible patients were enrolled in our study (10% of parents did not give consent mostly because they were living too far away from the clinic) and this could have introduced a bias in our results.

In conclusion, we showed that while HRSV remains a major cause of ARTI in children receiving palivizumab prophylaxis, the clinical outcome of the 10 HRSV-infected children we studied was generally mild. Future work is required to further evaluate the development of HRSV infections in children receiving multiple doses of palivizumab and to determine the additional benefits of more potent antibodies.

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