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ORIGINAL ARTICLE

Respiratory Syncytial Virus and Recurrent Wheeze in Healthy Preterm Infants

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ABSTRACT

BACKGROUND

Respiratory syncytial virus (RSV) infection is associated with subsequent recurrent wheeze. Observational studies cannot determine whether RSV infection is the cause of recurrent wheeze or the first indication of preexistent pulmonary vulnerability in preterm infants. The monoclonal antibody palivizumab has shown efficacy in preventing severe RSV infection in high-risk infants.

METHODS

In the double-blind, placebo-controlled MAKI trial, we randomly assigned 429 otherwise healthy preterm infants born at a gestational age of 33 to 35 weeks to receive either monthly palivizumab injections (214 infants) or placebo (215 infants) during the RSV season. The prespecified primary outcome was the total number of parent-reported wheezing days in the first year of life. Nasopharyngeal swabs were taken during respiratory episodes for viral analysis.

RESULTS

Palivizumab treatment resulted in a relative reduction of 61% (95% confidence interval, 56 to 65) in the total number of wheezing days during the first year of life (930 of 53,075 days in the RSV-prevention group [1.8%] vs. 2309 of 51,726 days [4.5%] in the placebo group). During this time, the proportion of infants with recurrent wheeze was 10 percentage points lower in patients treated with palivizumab (11% vs. 21%, $P=0.01$).

CONCLUSIONS

In otherwise healthy preterm infants, palivizumab treatment resulted in a significant reduction in wheezing days during the first year of life, even after the end of treatment. These findings implicate RSV infection as an important mechanism of recurrent wheeze during the first year of life in such infants. (Funded by Abbott Laboratories and by the Netherlands Organization for Health Research and Development; MAKI Controlled Clinical Trials number, ISRCTN73641710.)

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ILLNESS OF THE LOWER RESPIRATORY TRACT that is caused by respiratory syncytial virus (RSV) is the most common cause of hospital admission in the winter season during the first year of life.¹ Severe RSV bronchiolitis has been associated with an increase in subsequent rates of early wheezing,^{2,3} asthma, and possibly allergic sensitization later in life.⁴⁻⁷ Early childhood wheeze after RSV infection has a high prevalence, influences quality of life, and generates substantial health care costs.⁸⁻¹¹

The pathogenesis of recurrent wheeze after RSV infection is still poorly understood. Gern and Busse distinguished two nonexclusive relationships between RSV infection and wheezing.¹² First, RSV bronchiolitis may interfere with normal lung development or immune maturation and subsequently cause recurrent episodes of wheezing. Second, RSV infection may be the earliest stimulus for wheezing in children who are predisposed to wheeze by genetic susceptibility or preexisting abnormal lung function at birth. A birth cohort study provided limited evidence for a causal relationship between RSV and recurrent wheeze, since the timing of birth in relationship to the annual winter RSV peak predicted the risk of recurrent wheeze.¹³ So far, the potential causal role of RSV infection in the development of recurrent wheeze is debated, but strong empirical evidence is lacking.^{14,15} Wu and Hartert therefore concluded that a randomized clinical trial using RSV prophylaxis was warranted to confirm a causal relationship between RSV infection and recurrent wheeze.¹⁶

We performed the multicenter, double-blind, randomized, placebo-controlled MAKI trial to investigate the potential causal role of RSV infection in the pathogenesis of wheezing illness during the first year of life, using the commercially available monoclonal antibody palivizumab (Synagis, MedImmune) against RSV.

METHODS

PATIENTS

From April 2008 through December 2010, we enrolled preterm infants (gestational age, 33 to 35 weeks) in pediatric departments of one university and 15 regional hospitals in the Netherlands. All the infants were otherwise healthy and 6 months of age or younger at the start of the RSV season. We excluded infants with congeni-

tal heart disease, bronchopulmonary dysplasia, Down's syndrome,¹⁷ or other serious congenital disorders and infants who required mechanical ventilation at birth, who were treated with surfactant, or who had physician-diagnosed wheeze before the start of the RSV season.

Parents provided written informed consent for study participation. The study was conducted according to the principles of the Declaration of Helsinki (version 2000). A yearly monitoring program that followed current Good Clinical Practice guidelines was run routinely.

ETHICAL ISSUES

Palivizumab is registered but not reimbursed in the Netherlands for preterm infants born at a gestational age of 33 to 35 weeks. Because RSV immunoprophylaxis is effective in preventing RSV lower respiratory tract illness in such preterm infants,¹⁸ our study was marked as a therapeutic study. The institutional review board at the University Medical Center Utrecht decided that the 50% chance of benefit of RSV prevention with palivizumab outweighed the risk of moderate side effects caused by the intramuscular administration of placebo and the burden of participating in this trial. The protocol was reviewed and approved by the institutional review board at the University Medical Center Utrecht and at each participating hospital.

RANDOMIZATION

Eligible infants were randomly assigned in a 1:1 ratio to receive either palivizumab (at a dose of 15 mg per kilogram of body weight) or placebo during the winter season (details are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org). The blinding of study-group assignment was performed with a randomization list that used a permuted-block design, which was generated by an independent pharmacist before the start of the trial. The randomization was stratified according to gestational age. Blinding was achieved with the use of a placebo matching the reconstituted palivizumab solution. The researchers who received the logs and performed the analyses and the parents were unaware of study-group assignments until 1 year of follow-up was completed for all participants. The research nurses who administered the study drugs were aware of study-group assignments because it was not feasible to prepare and administer the

treatment in a blinded fashion within 3 hours after reconstitution. The research nurses were trained to reveal no knowledge of the randomization to parents and were not involved in the reporting of data analyses. The research nurses worked with standard operating procedures and were carefully instructed to prevent possible unblinding.

STUDY OUTCOMES AND FOLLOW-UP

The primary outcome was number of parent-reported wheezing days in the first year of life. Using methods identical to those used in our previous trial, parents recorded airway symptoms, doctor visits, and the use of airway drugs in a daily log until their infant was 1 year of age.^{19,20} Instructions for completing the log were given during the first home visit, and compliance was checked at each subsequent home visit.

Secondary outcomes were the number of days with bronchodilator use, the number of RSV infections confirmed by means of a nasopharyngeal swab positive for RSV RNA with or without medical attention, the number of hospitalizations for laboratory-proven RSV infection, the number of wheezing episodes, and the prevalence of recurrent wheeze. Medical attention was defined as a visit to either a general practitioner or a hospital. A wheezing episode was defined as a respiratory episode with wheezing on more than 1 day. The interval between two episodes was defined as a period of at least 7 days without respiratory symptoms. Recurrent wheeze was defined as three or more episodes of wheezing during the first year of life. A family history of atopy was defined as a physician diagnosis of asthma, hay fever, or eczema in at least one of the parents.

LABORATORY TESTS AND FOLLOW-UP

We defined the post-prophylaxis period as the follow-up from 2 months after the last treatment administration (three half-lives of palivizumab) up to the age of 1 year. In case of respiratory symptoms, primary care was left to the general practitioner.

Parents were instructed to take a nasopharyngeal swab in case of the occurrence of respiratory symptoms with involvement of the upper or lower respiratory tract lasting more than 1 day. The swab was transported in a viral transport medium by regular mail to the laboratory and stored at -80°C until polymerase-chain-reaction (PCR) assays were performed. The presence of

RSV RNA was determined by multiplex real-time reverse-transcriptase-PCR with the use of previously published primers and probes for RSV-B²¹ and primers and probes for RSV-A that were developed in-house (details are provided in the Supplementary Appendix).

We determined the presence of 16 respiratory viruses and 4 respiratory bacteria using the RespiFinder SMART 22 assay (PathoFinder).²² Positive results on testing for rhinovirus or enterovirus are referred to as rhinovirus infection. All hospitalizations were evaluated, and any deaths were regarded as serious adverse events. Local injection-site reactions and physician visits for nonrespiratory symptoms were not recorded.

STUDY OVERSIGHT

The academic authors designed and conducted the study without input from the study sponsor (Abbott Laboratories, which markets palivizumab) other than financial support and donation of the palivizumab. All authors vouch for the accuracy and completeness of the data reported and for the fidelity of this report to the study protocol, available at NEJM.org.

STATISTICAL ANALYSIS

The sample-size calculation was based on a clinically relevant between-group difference of a mean (\pm SD) of 5 ± 15 wheezing days during the first year of life.^{20,23,24} The predefined target of 226 infants per study group provided a power of at least 90% to detect a clinically relevant difference in wheezing days with the use of an alpha level of 0.05. Since a typical Poisson distribution for probability arose, we used Poisson regression analysis to study potential differences in the number of days with wheeze.²⁰ Percentages and associated 95% confidence intervals of infants with wheezing or recurrent wheeze episodes were calculated. We used chi-square tests, Student's t-tests, and Mann-Whitney U tests to evaluate differences in percentages, mean values, and median values between the two study groups. All analyses were performed on an intention-to-treat basis. No imputation of missing data was performed, since the overall amount of missing data was less than 10%. Post hoc subgroup analyses were performed to assess wheezing days in subgroups of children with a family history of atopy or asthma. All statistical analyses were performed with SPSS software, version 20.0.

RESULTS

PATIENTS

Of 1550 late preterm infants (gestational age, 33 to 35 weeks) who underwent screening, 429 were enrolled in the study (Fig. 1A). The median day of birth was August 22 for infants who were enrolled in the study, as compared with a median day of birth of August 5 for infants who were not enrolled. The two study groups were well balanced on the basis of inclusion year, gestational age, and birth month. Birth weight, family history of atopy, presence of siblings, and other baseline characteristics were similar, except for sex (58% male infants in the RSV-prevention group vs. 44% in the placebo group) (Table S1 in the Supplementary Appendix). By design, only children with no previous wheezing symptoms were enrolled.

A median number of 4 injections during the RSV season were administered to infants in the RSV-prevention group (range, 1 to 5) and the placebo group (range, 2 to 5). In the RSV-prevention group, 95% of scheduled injections and 89% of follow-up of daily logs were completed, as compared with rates of 92% and 88%, respectively, in the placebo group. The median follow-up duration was 10 months (range, 0 to 12) in the two study groups.

RSV INFECTION

We studied the occurrence and severity of RSV infection to confirm the efficacy of RSV immunoprophylaxis in our study population. We confirmed that infants who were treated with palivizumab had a lower incidence of RSV-related hospitalization than those treated with placebo (0.9% vs. 5.1%, $P=0.01$).¹⁸ The infants who were treated with palivizumab also had a lower incidence of medically attended nonhospitalized RSV infection (Table 1).

PRIMARY AND SECONDARY OUTCOMES

The number of days with parent-reported wheeze was lower in the RSV-prevention group than in the placebo group (Table 2 and Fig. 2). This result was consistent for all 3 study years and independent of the number of injections of palivizumab or placebo. There was an absolute reduction of 2.7 percentage points in rates of wheezing in the RSV-prevention group versus the placebo group (930 of 53,075 days [1.8%] and 2309 of 51,726 days [4.5%], respectively), for a relative reduction of 61% (95% confidence interval [CI], 56 to 65).

The effect of RSV prevention on the number of wheezing days persisted during the post-prophylaxis period (i.e., starting at 2 months after the last injection), for a relative reduction of 73% (95% CI, 66 to 80). Similarly, there was a decrease in the number of wheezing days outside the RSV season in the RSV-prevention group (Table 2). Among children with any proven RSV infection, there was no significant between-group difference in the incidence of wheezing (23% in the RSV-prevention group and 30% in the placebo group) or in the mean number of wheezing days during the first year of life (8.2 days in the RSV-prevention group and 16 days in the placebo group). We did not detect RSV reinfection in either group.

The proportion of infants with recurrent wheezing was lower in the RSV-prevention group than in the placebo group (11.2% vs. 20.9%, $P=0.005$) (Table 3). Similarly, the proportion of infants using bronchodilators was lower in the RSV-prevention group than in the placebo group (13% vs. 23%, $P<0.001$). The effect of RSV prevention on the total number of wheezing days was not significantly different ($P=0.89$) in children without a family history of atopy (72% reduction; 95% CI, 65 to 79), as compared with those with a family history of atopy (54% reduction; 95% CI, 47 to 60). A similar effect of RSV prevention was seen in children without and with parental asthma (68% reduction [95% CI, 62 to 73] vs. 35% reduction [95% CI, 23 to 47]). The total numbers of respiratory episodes were similar in the two study groups. However, we found more coinfections during nonwheezing episodes in the RSV-prevention group than in the placebo group (101 of 236 swabs [43%] vs. 63 of 197 swabs [32%], $P=0.02$) (Fig. 1B).

ADVERSE EVENTS

The proportion of patients with serious adverse events was lower in the RSV-prevention group than in the placebo group. We observed 32 hospitalizations in 27 children (12.6%) in the RSV-prevention group, as compared with 52 hospitalizations in 47 children (21.9%) in the placebo group ($P=0.04$). Reasons for hospitalization in the RSV-prevention group were RSV infection (in 2 patients), other respiratory tract illness (in 6), gastroenteritis (in 6), surgery (in 6), failure to thrive (in 6), and other reasons (in 6). Reasons for hospitalization in the placebo group were RSV

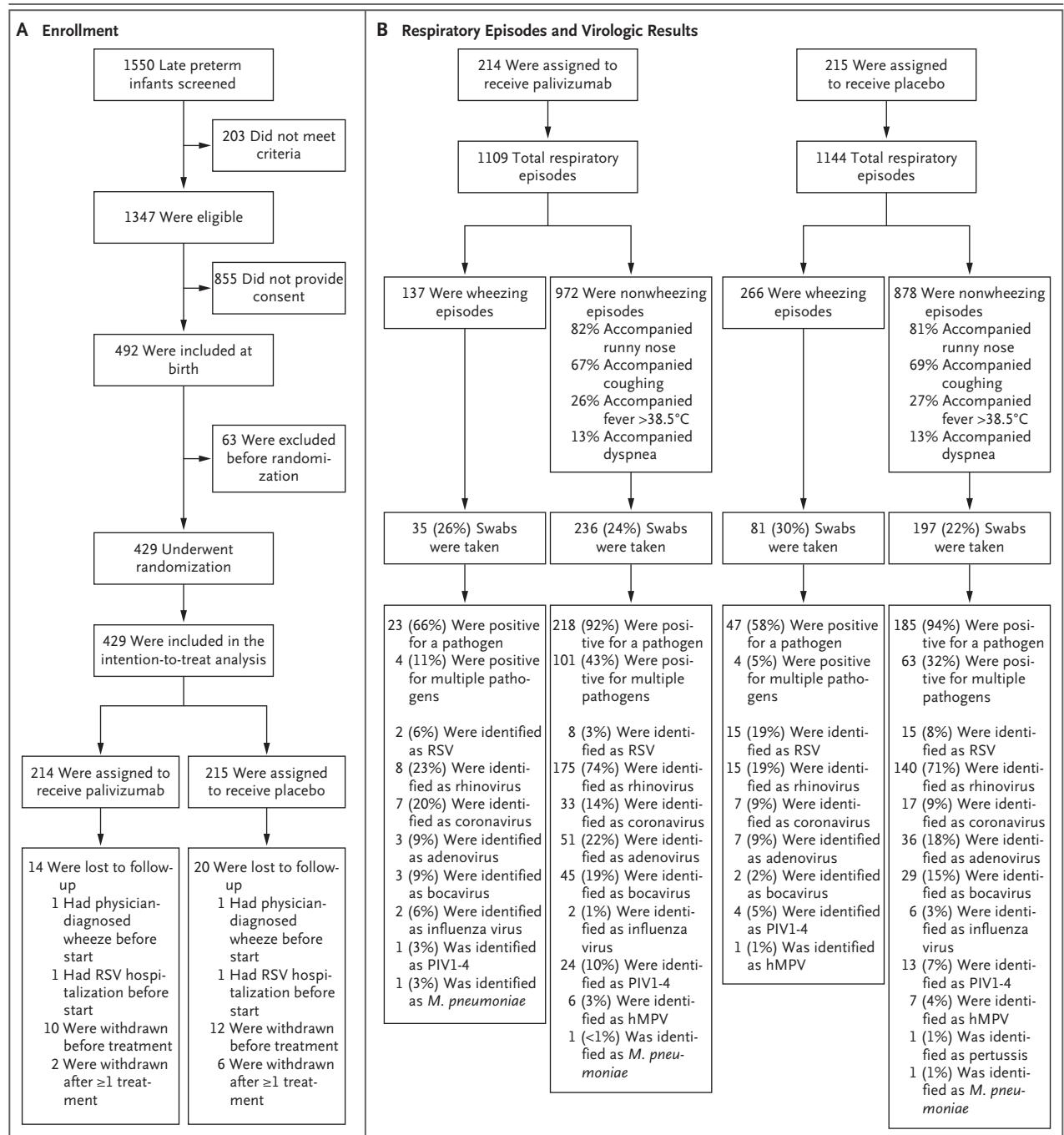


Figure 1. Enrollment, Number of Respiratory Episodes, and Results of Virologic Analyses.

Panel A shows enrollment and study outcomes for the 429 infants who were included in the intention-to-treat analysis. Panel B shows the total number of respiratory symptoms, which were based on parent records. A respiratory episode was defined as an episode of at least 2 consecutive days of upper or lower respiratory symptoms. Parents were instructed to take a nasopharyngeal swab on the second day of every respiratory episode. Respiratory syncytial virus (RSV) was detected with the use of in-house real-time reverse-transcriptase–polymerase-chain-reaction assays, and the RespiFinder SMART 22 assay was used for the detection of adenovirus, bocavirus, *Bordetella pertussis*, *Chlamydia pneumoniae*, coronavirus (229E, HKU1, NL63, and OC43), human metapneumovirus (hMPV), influenza virus type A, influenza virus A(H1N1)pdm09, influenza virus type B (influenza virus), *Legionella pneumophila*, *Mycoplasma pneumoniae*, parainfluenza virus types 1 through 4 (PIV1-4), RSV types A and B (RSV), and rhinovirus or enterovirus (rhinovirus).

infection (in 11 patients), other respiratory tract illness (in 6), gastroenteritis (in 10), surgery (in 13), failure to thrive (in 8), and other reasons (in 4). There were no deaths.

DISCUSSION

In this proof-of-concept study, treatment with a monoclonal antibody for RSV prevention in late preterm infants greatly reduced the number of parent-reported wheezing days during the first year of life, even after the end of therapy and outside the RSV season. RSV prevention reduced wheezing, but wheezing was not eliminated. RSV prevention was associated with a relative reduction of 61% in the number of wheezing days, a finding that shows that RSV infection is an im-

portant mechanism in the pathogenesis of wheezing morbidity in this specific population.

Our results are in line with other studies that acknowledge the relationship between RSV bronchiolitis and recurrent wheeze.^{4,7,9,25-27} Wu et al.¹³ found that the timing of birth date with respect to the peak of the winter bronchiolitis season was related to the risk of asthma. These findings suggest that asthma is most likely to develop in infants who are at highest risk for severe viral bronchiolitis. However, other studies have argued against RSV as the cause of pulmonary damage and subsequent early childhood wheezing.² The role of RSV in the development of asthma remains controversial, and our data cannot provide evidence in this discussion.²⁸ A previous non-randomized trial suggested that the prevention

Table 1. Proportion of Infants with Proven Respiratory Syncytial Virus (RSV) Infection.*

Variable	Palivizumab (N=214)	Placebo (N=215)	Absolute Risk Reduction†	Relative Risk Reduction (95% CI)‡	P Value
	no. (%)	no. (%)	percentage points	%	
Total RSV infection	10 (4.7)	30 (14.0)	9.3	67 (27 to 107)	0.001
Hospitalization for RSV infection	2 (0.9)	11 (5.1)	4.2	82 (18 to 157)	0.01
Medically attended RSV infection without hospitalization	2 (0.9)	10 (4.7)	3.7	80 (11 to 161)	0.02
RSV infection without medical attention	6 (2.8)	9 (4.2)	1.4	33 (-56 to 126)	0.40

* Medical attention was registered during the home visits and reported by parents on the daily log.

† The absolute and relative values for risk reduction are for the palivizumab group as compared with the placebo group.

Table 2. Days with Wheezing in the First Year of Life.*

Variable	Palivizumab (N=214)			Placebo (N=215)			Absolute Reduction†	Relative Risk Reduction (95% CI)‡
	Total Log Days	Total Symptom Days	Incidence per Day	Total Log Days	Total Symptom Days	Incidence per Day	no. of symptom days	%
	no.	no.	%	no.	no.	%		
Days with wheezing								
First year of life	53,075	930	1.8	51,726	2309	4.5	1379	61 (56-65)
<2 mo after prophylaxis	28,455	666	2.3	28,220	1382	4.9	716	52 (46-59)
≥2 mo after prophylaxis	24,620	264	1.1	23,506	927	3.9	663	73 (66-80)
During RSV season‡	26,176	646	2.5	26,081	1348	5.2	702	52 (46-59)
Outside RSV season‡	26,899	284	1.1	25,645	961	3.7	677	73 (66-80)

* The incidence of wheezing was calculated as the number of days with parent-reported airway symptoms divided by the number of log days during follow-up. P<0.001 for all comparisons except P=0.006 for the category of less than 2 months after the end of prophylaxis.

† The values for absolute reduction and relative risk reduction are for the palivizumab group as compared with the placebo group.

‡ The RSV season was defined as October 1 to March 31.

of lower respiratory tract illness caused by RSV reduced subsequent recurrent wheeze in infants without a family history of atopy but showed no effect in infants with a family history of atopy.^{29,30} We found that RSV prevention was associated with reduced wheezing in the first year of life, regardless of whether there was a family history of atopy. Our study underlines the important role that RSV plays in the pathogenesis of recurrent wheeze. We hypothesize that RSV primarily causes direct pulmonary epithelial damage and local immunologic alterations in the lungs, leading to long-term airway hyperresponsiveness and wheezing.

A study in mice showed that RSV causes persistent airway hyperresponsiveness, chronic lung inflammation, and histopathological abnormalities.^{31,32} Altered immune-response patterns have been described after RSV infection. Studies in mice and humans have suggested that local production of interleukin-10 during RSV infection is a key mechanism in the development of recurrent wheeze and airway hyperresponsiveness, although mechanisms independent of interleukin-10 have also been described.^{19,33-36} We believe that alterations to the pulmonary environment and immunologic phenotype caused by RSV infection in early life eventually lead to long-term remodeling of the pulmonary system and hyperresponsiveness to respiratory viruses and nonspecific stimuli.

In our study, the numbers of respiratory episodes were similar in the two study groups. However, in the RSV-prevention group, we found more coinfections than in the placebo group. Previous studies have not addressed the effect of palivizumab on the acquisition or clearance of respiratory viruses other than RSV. RSV bronchiolitis is followed by a robust inflammatory response in the airways, which may persist for more than

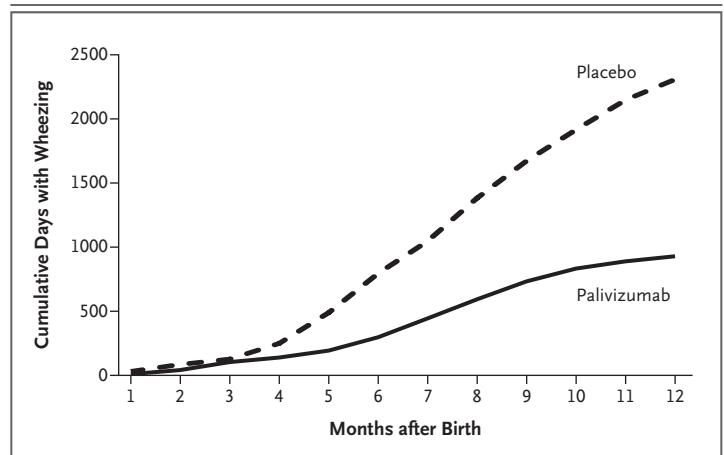


Figure 2. Cumulative Wheezing Days for 429 Preterm Infants during the First Year of Life.

$P < 0.001$ for the comparison between palivizumab and placebo with the use of Poisson regression.

1 month.³⁷ We speculate that this inflammatory response, including production of interferons, transiently protects against subsequent viral infection, resulting in fewer coinfections.^{38,39} More research is needed to unravel how respiratory viruses interact at the mucosal level.

The major strength of our study is the randomized design, which precludes bias from selection or confounding and which subsequently provided unbiased and conclusive evidence regarding the mechanism of RSV infection in the pathogenesis of infant wheezing. Some potential limitations should also be discussed. First, parents with an atopic history may have been more likely to participate in the study. However, since the stratified results did not differ between infants of parents with and those without an atopic history, our conclusions are generalizable. Second, although nasopharyngeal swabs were ob-

Table 3. Infants with Wheezing.*

Variable	Palivizumab (N=214)	Placebo (N=215)	Absolute Reduction†	Relative Risk Reduction (95% CI)‡
Any wheezing — no. of infants (%)	66 (30.8)	101 (47.0)	16.2	34 (14–53)
Wheezing episodes — no.	137	266	129	48 (32–62)
Recurrent wheezing — no. of infants (%)	24 (11.2)	45 (20.9)	9.7	47 (14–80)

* Any wheezing was defined as at least one episode of wheezing during the first year of life. A wheezing episode was defined as a respiratory episode with wheezing on more than 1 day. Recurrent wheezing was defined as three or more episodes of wheezing during the first year of life. $P < 0.001$ for the between-group comparisons for any wheezing and wheezing episodes and $P = 0.005$ for recurrent wheezing.

† The values for absolute reduction are percentage points, and the values for relative risk reduction are numbers of episodes.

tained by parents to increase compliance of sampling,⁴⁰ swabs were obtained in approximately 30% of all respiratory episodes. This is similar to the range of percentages (24 to 43%) obtained in a study with a similar approach to parental swab collection.^{40,41} Consequently, we may underestimate the incidence of RSV infection. However, since the trial was double-blind and randomized, we do not believe this factor had an effect on the overall conclusions. Third, preterm infants are at higher risk for recurrent episodes of wheezing than are term infants.⁴² Therefore, we do not know whether our results can be generalized to healthy term infants. Fourth, we had to rely on parent-reported morbidity data, since no objective measure of wheezing was available. Identifying wheezing is problematic even for trained clinicians.^{43,44} However, since the parents were unaware of study-group assignments, we believe that any misclassification of wheezing was random in the two groups.

In summary, we have shown that the administration of palivizumab for RSV prevention reduced the total number of wheezing days in the first year of life among preterm infants with a gestational age of 33 to 35 weeks. The post-

prophylaxis effect of RSV prevention on wheezing illness is evidence that RSV infection is an important mechanism in the pathogenesis of wheezing during the first year of life among late preterm infants.

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