

Detection of IgA and IgG but not IgE antibody to respiratory syncytial virus in nasal washes and sera from infants with wheezing

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Background and Objective: The capacity of respiratory syncytial virus (RSV) to stimulate an IgE antibody response and enhance the development of atopy and asthma remains controversial. Nasal washes and sera from 40 infants (20 with wheezing, 9 with rhinitis, and 11 without respiratory tract symptoms) were obtained to measure IgE, IgA, and IgG antibody to the immunodominant, F and G, virion proteins from RSV.

Study design: Children (aged 6 weeks to 2 years) were enrolled in the emergency department during the mid-winter months and seen at follow-up when they were asymptomatic. All nasal washes were tested for RSV antigen. Determinations of antibody isotypes (IgE, IgA, and IgG) to RSV antigens were done in nasal washes and sera by using an enzyme-linked immunosorbent assay. In a subset of nasal washes, IgE to RSV was also evaluated by using a monoclonal anti-F_cE antibody-based assay.

Results: Fifteen patients with wheezing, two with rhinitis, and one control subject tested positive for RSV antigen at enrollment. Thirteen patients with wheezing were <6 months old, and most (77%) were experiencing their first attack. Among the children with positive test results for RSV antigen, an increase in both nasal wash and serum IgA antibody to RSV-F_a and G_a was observed at the follow-up visit. However, there was no evidence for an IgE antibody response to either antigen.

Conclusion: Both IgA and IgG antibodies to the immunodominant RSV-F_a and G_a antigens were readily detected in the nasal washes and sera from patients in this study. We were unable to demonstrate specific IgE antibody to these antigens and conclude that the production of IgE as a manifestation of a T_H2 lymphocyte response to RSV is unlikely. (J Pediatr 2001;138:311-7)

During the first 2 years of life, most attacks of wheezing are caused by the respiratory syncytial virus.¹⁻³ Although the majority of infants who wheeze with RSV can be treated at home, about 1% require hospitalization for moderate to severe illness, predominantly during the first 6 months of life.¹ Several studies suggest that RSV bronchiolitis may be a risk factor for recurrent wheezing and the development of asthma during childhood.⁴⁻⁶ However, mechanisms explaining how RSV might influence this process continue to be investigated and remain controversial. Although there has been speculation that viral respiratory tract infections early in life may facilitate sensitization to aeroallergens,⁶⁻⁸ long-term studies have failed to show a relationship between RSV wheezing in infancy and the development of atopy.⁹⁻¹² In addition, recent epidemiologic studies raise the possibility that respiratory tract infections in early childhood may protect against asthma and atopy.^{13,14}

ABU	Antibody-binding units
ELISA	Enzyme-linked immunosorbent assay
RSV	Respiratory syncytial virus

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In favor of the possibility that RSV-induced wheezing may predispose infants to develop asthma are reports that RSV may have the capacity to stimulate a T_H2 lymphocyte response.^{15,16} Mice immunized with the RSV G (attachment) protein were shown to develop pulmonary eosinophilia after they were subsequently infected with

Table I. Patient characteristics

	Wheezing group (n = 20)	Rhinitis group (n = 9)	Control group (n = 11)
African American*	11 (55)	5 (56)	5 (45)
Male*	13 (65)	6 (67)	8 (73)
Family history of allergy* [†]	7 (35)	1 (11)	0 (0)
Exposure to tobacco smoke in home* [‡]	14 (70)	8 (89)	7 (64)
Present at follow-up visit*	19 (95)	7 (78)	7 (64)
Age (mo)			
Median	5.5	7.0	10.0
Range	1-18	2-12	6-21
Total serum IgE (IU/mL)			
Median	6.6	3.5	2.6
25th, 75th percentiles	2.2, 14.3	1.9, 7.8	2.1, 13.0

*Values are expressed as number of patients, followed in parentheses by the percentage of patients enrolled within each study group.

[†]History of asthma, hay fever, eczema, or food hypersensitivity in the immediate family (parents and siblings).

[‡]Exposure to environmental tobacco smoke from one or more smokers in the home determined from questionnaires.

RSV intranasally.^{17,18} Evidence for an IgE-mediated response in humans was based on the detection of IgE antibody to RSV in nasal secretions from infants who were hospitalized with RSV bronchiolitis. In these studies, the detection of IgE antibody to RSV was reported in response to cell surface antigen, to sucrose gradient-purified RSV, and subsequently to affinity-purified RSV F and G envelope antigens.¹⁹⁻²¹ However, these results have been difficult for others to reproduce. In the present study, levels of IgA, IgG, and IgE antibody to the immunodominant RSV F (fusion) and G (attachment) antigens were compared in nasal washes and sera from infants who were enrolled in the study in our emergency department.

METHODS

Study Population

Forty children, aged 6 weeks to 2 years, were evaluated in the University of Virginia Pediatric Emergency Department from December 1994 through April 1997. Eighty-three percent were enrolled during the months

of December through March when RSV infections are common in central Virginia. In all 20 of the children with chest symptoms, wheezing by auscultation was documented by physicians in the emergency department, and they all received treatment with at least an inhaled β_2 -agonist. Infants with a history consistent with the diagnosis of bronchopulmonary dysplasia or congenital heart or lung disease were not included. Nine infants with rhinitis but no lower respiratory tract symptoms and 11 children without any respiratory tract symptoms were also enrolled. The children without respiratory tract symptoms were brought to the emergency department primarily because of minor trauma, rashes, and gastrointestinal complaints. Thirty-three patients (82.5%) were also seen for follow-up when they were asymptomatic. Most of them (88%) were seen at follow-up 4 to 9 weeks after their enrollment (median, 6 weeks; range, 4-28 weeks). With the use of methods previously described, sera and nasal washes were obtained from patients both at the enrollment and the follow-up

visit.²² Demographic information was obtained from each patient's chart and from a questionnaire that was administered to parents at the initial visit. This study was approved by the Human Investigation Committee at the University of Virginia, and informed written consent was obtained from parents.

RSV Antigens

Monoclonal antibody affinity-purified preparations of the F (fusion) and G (attachment) antigens from RSV were used in these experiments. Their purification and physicochemical characterization have been described previously.^{23,24} The 70-kd F glycoprotein (RSV-F_a) was isolated from the RSV A strain and is structurally homologous and antigenically similar to the F protein from the B strain of RSV (RSV-F_b).²⁵ The 90-kd G glycoprotein is heavily glycosylated. The G proteins from the A and B subtypes of RSV have shown antigenic diversity in binding human antibody.²⁴ For this reason, G proteins from both the RSV A and B strains were used in the assessment of antibody to RSV in the nasal wash experiments.

Immunoassays

Supernatants from nasal washes and serum from the blood samples were stored at -70°C for subsequent measurements of antibody to the RSV antigens. The RSV status of each patient was determined by testing for RSV antigen in nasal wash fluid with a membrane enzyme immunoassay (Abbott Diagnostics Division, Chicago, Ill). Measurements of total serum IgE were done by using a fluorometric immunoassay (CAP system) developed by Pharmacia Diagnostics, Uppsala, Sweden.

Antibody isotype responses (IgG, IgA, and IgE) to the RSV F_a, G_a, and G_b antigens were measured in an enzyme-linked immunosorbent assay as previously described.²⁵ In previous studies of IgE antibody to RSV, blocking antigen-coated plates to reduce non-specific binding of antibody in

nasal washes was not described.¹⁹⁻²¹ In the present study, Immulon No. 2 flat bottom ELISA plates coated with F_a , G_a , or G_b proteins were blocked with 1% bovine serum albumin in phosphate-buffered saline–1% Tween before the addition of serum (diluted 1:3 and 1:9) or nasal washes (diluted 1:2 and 1:4) in the same blocking solution. Bound antibody was detected by using biotin-labeled goat anti-IgG, anti-IgA, or anti-IgE antibody (Kirkegaard and Perry Laboratories, Gaithersburg, Md). Control curves were established for IgG and IgA antibody binding to F_a , G_a , and G_b by using pooled serum from 3 adults (age >24 years) who were naturally sensitized to the RSV antigens. Maximal binding of IgG antibody to F_a was obtained by using a 1:100 dilution of pooled serum, which was assigned a value of 10 antibody-binding units/mL (ie, 1000 binding units/mL of undiluted pooled serum). Titers of antibody in samples were determined separately from control curves for IgG and IgA by using starting dilutions of serum pool of 1:25 for IgG to G_a and G_b and 1:5 for IgA to F_a , G_a , and G_b . Differences in control curve slopes were examined with the Wald test for interaction between antibody isotype and the natural logarithm of ABU/mL after fitting least-squares linear regression lines. No significant difference between the slopes for IgG and IgA binding to RSV- F_a was detected ($P = .97$). Thus the titers of IgA and IgG antibody to the F_a antigen in patient samples should be comparable. Similarly, no significant difference was detected between the slopes for IgG and IgA binding to RSV- G_a ($P = .94$) or to RSV- G_b ($P = .88$).

The presence of IgE antibody to the RSV F and G antigens was also evaluated by using a monoclonal antibody-based assay. This assay was done with monoclonal anti-IgE antibody (CIA/E/7.12 and CIA/E/4.15), kindly provided by Dr Andrew Saxon, University of California, Los Angeles.²⁶ In this assay, cyanogen bromide-activated

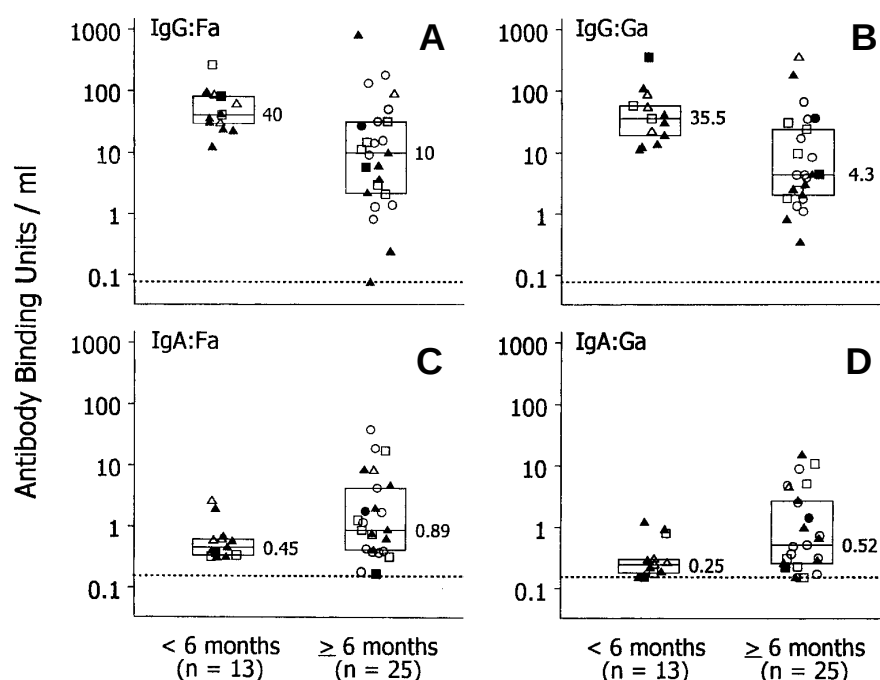


Fig 1. IgG and IgA antibody to RSV- F_a and G_a antigens in sera obtained from patients at enrollment in the emergency department. Patients with wheezing (triangles), patients with rhinitis (squares), and control patients (circles) are indicated along with their RSV status (solid symbols indicate patients who tested positive for RSV; open symbols indicate patients with negative test results). **Panels A and B** show results for IgG binding to F_a and G_a , respectively. **Panels C and D** show results for IgA binding to F_a and G_a . Boxes indicate the interquartile range for each patient group. The median is indicated by the line within each box, and its value is printed outside the box to the right. The median values for IgG antibody to RSV- F_a and G_a among infants <6 months old were significantly higher than values from children 6 months of age or older (**A and B**: $P = .004$ and $.002$, respectively). Median values for IgA antibody to RSV- F_a and G_a were significantly lower among the children who were <6 months old (**C and D**: $P = .04$ and $.03$, respectively).

disks coated with monoclonal anti-IgE were incubated with nasal wash supernatant (diluted 1:2 and 1:4 in 1% bovine serum albumin–phosphate-buffered saline Tween), and iodine 125-labeled affinity-purified F_a or G_a antigen was used to detect bound antigen-specific IgE antibody.

Statistical Analysis

The 25th, 50th (median), and 75th percentiles were used to describe the distribution of antibody titers within patient groups (eg, RSV-positive and RSV-negative patients). The Wilcoxon signed rank test was used to compare 2 groups of paired antibody titers (eg, enrollment vs follow-up), and the Wilcoxon rank sum test was used to test for differences between 2 unpaired groups.

RESULTS

Patient Characteristics

Among the 40 children enrolled in this study, positive test results for RSV antigen were detected in nasal washes from 75% (15 of 20) of the children with wheezing, 22% (2 of 9) of those with rhinitis, and 9% (1 of 11) of control subjects at the time of enrollment in the emergency department. Ten of the 20 patients with wheezing were <6 months old and were experiencing their first episode of RSV bronchiolitis according to their history and chart review. Twelve of the infants with wheezing (60%) were hospitalized; 11 of the 12 tested positive for RSV, and 9 were <6 months of age. Five patients with wheezing who tested positive for

Table II. IgA and IgG antibody to RSV-F_a and RSV-G_a antigens in sera and nasal washes at enrollment and follow-up

	F _a		G _a	
	Enrollment	Follow-up	Enrollment	Follow-up
Serum				
IgA: RSV-positive (n = 13)	0.57 (0.40, 0.89)	2.22 (1.05, 7.48)*	0.24 (0.16, 0.74)	4.41 (0.53, 15.9) [†]
IgA: RSV-negative (n = 15)	0.88 (0.38, 5.40)	0.48 (0.39, 7.25)	0.30 (0.21, 4.66)	0.57 (0.17, 2.75)
IgG: RSV-positive (n = 13)	12.3 (3.60, 41.4)	127 (58.0, 242) [†]	7.85 (2.40, 41.8)	167 (52.9, 247)*
IgG: RSV-negative (n = 15)	29.5 (12.9, 55.6)	45.4 (8.10, 68.3)	22.0 (4.34, 35.1)	14.2 (5.15, 25.8)
Nasal wash				
IgA: RSV-positive (n = 15)	1.06 (0.40, 2.35)	2.31 (0.95, 5.13)	1.15 (0.36, 1.70)	2.25 (1.24, 3.70) [‡]
IgA: RSV-negative (n = 18)	1.25 (0.49, 1.88)	2.10 (0.59, 4.00) [‡]	0.94 (0.41, 2.67)	0.94 (0.41, 2.28)
IgG: RSV-positive (n = 15)	0.24 (0.14, 0.67)	0.81 (0.33, 3.10) [‡]	0.25 (0.07, 0.43)	0.22 (0.09, 0.66)
IgG: RSV negative (n = 18)	0.29 (0.12, 1.37)	0.19 (0.08, 0.46)	0.12 (0.06, 0.22)	0.06 (0.06, 0.07)

Values are expressed as the median (ABU/mL) with 25th and 75th percentiles in parentheses. To permit statistical comparisons, only data for patients who were seen both at enrollment and at follow-up are included. Data from clinical groups (ie, wheezing, rhinitis, and control) were pooled because no significant differences were observed between the groups.

Values at follow-up that are significantly different from values at enrollment are indicated by symbols.

**P* < .001.

[†]*P* < .01.

[‡]*P* < .05.

RSV at enrollment still had positive test results at follow-up. All other patients tested negative for RSV at follow-up, except for one control patient who had a negative test result at enrollment. Although total serum IgE levels from infants with wheezing were higher than the levels from infants with rhinitis or control subjects, the difference was not significant (Table I).

Antibody to RSV F_a and G_a Antigens in Serum

Among infants <6 months of age, who are more likely to have maternally acquired IgG antibody in their sera, the levels of serum IgG antibody to both RSV-F_a and G_a antigens at enrollment were significantly higher than in sera from older children (Fig 1, *A* and *B*). In contrast, their levels of IgA antibody to these antigens were substantially lower (Fig 1, *C* and *D*). In the emergency department, IgG and IgA antibody titers to F_a or G_a were not strikingly different in sera from children who tested positive for RSV compared with those who tested negative (Fig 1, Table II). However, a significant rise in the titers of IgA and IgG

antibody to both antigens was evident at follow-up in sera from those children who tested positive for RSV, but not from those who tested negative (Table II). The sera were also tested for IgE antibody to RSV-F_a and G_a antigens, but all values fell below the detection limits of the assays for the binding of both IgG and IgA antibody to these antigens (ie, 0.08 ABU/mL for IgG to RSV-F_a and G_a and 0.12 ABU/mL for IgA to RSV-F_a and G_a).

Antibody to RSV F_a and G_a Antigens in Nasal Washes

An increase in the titers of IgA and IgG antibody to the RSV F_a antigen (>2-fold for IgA and >3-fold for IgG antibody) was observed at the follow-up visit in nasal washes from RSV-positive patients (Fig 2, *A* and Table II). An increase in IgA, but not IgG antibody, to RSV-G_a was detected at follow-up in washes from patients who tested positive for RSV (Fig 2, *B*, and Table II). By comparison, the change in antibody titers to F_a and G_a in washes from the RSV-negative patients at follow-up was negligible, except for IgA antibody to F_a. This RSV-negative

group included a control patient who tested positive for RSV at the follow-up visit but who tested negative at enrollment. We speculate that most of the patients in this study may have been infected with the A strain of RSV, because the change in IgA and IgG antibody binding to the RSV-G_b antigen in washes from the RSV-positive patients at the follow-up visit was not significant (data not shown). The ratio of IgA/IgG antibody to both the RSV F_a and G_a antigens was substantially higher in nasal washes from RSV-positive patients (median ratios of 1.92 and 4.75 for F_a and G_a, respectively) than the ratio of these isotypes in sera (median ratios of 0.03 and 0.04 for F_a and G_a, respectively), indicating that the antibody responses detected in nasal washes are likely to reflect local antibody production without a significant contribution of antibody from vascular leakage.

In contrast to titers of IgA and IgG antibody, which were readily detected in response to RSV-F_a and G_a, the values for IgE antibody in most nasal washes were below the limits of the assays for detecting IgG and IgA to these antigens (Fig 2). Four infants had test

results that were positive, but were very close to the assay detection limits according to control curves for IgA to RSV- F_a and G_a . None of the 4 had a test result that was positive at both enrollment and follow-up. Three were RSV-positive infants with wheezing (ages 2, 5, and 12 months); 2 of them were hospitalized, and only 1 (the 5 month old infant) had wheezed previously. The 12-month-old infant had positive test results for IgE antibody to both F_a and G_a at enrollment. The fourth infant, who was 4 months old, tested negative for RSV and had rhinitis. Washes from these children were also analyzed by using a monoclonal anti-human IgE antibody on solid phase to capture IgE antibody in nasal wash supernatant, followed by incubation with ^{125} I-labeled F_a or G_a antigen. Nasal washes from the 2 patients with IgE binding to F_a in the ELISA, from the 3 patients with IgE binding to G_a , and from 2 patients who tested negative for IgE antibody to both antigens were included. No specific IgE antibody to either F_a or G_a was detected in this assay.

DISCUSSION

Prospective studies have shown that RSV bronchiolitis in infancy is associated with a higher risk for recurrent wheezing and pulmonary dysfunction during the school-age years.^{4,5} However, it is also true that most infants who wheeze with RSV no longer wheeze after their toddler years and are unlikely to develop asthma.^{27,28} It is in this context that the novel observation suggesting that RSV could stimulate IgE antibody stands out as an intriguing mechanism for wheezing among RSV-infected infants.¹⁹⁻²¹

In previous studies of IgE antibody to RSV, the subjects enrolled frequently included infants with wheezing who were hospitalized, most of whom were <6 months of age.^{19-21,29,30} According to our results, these infants are more

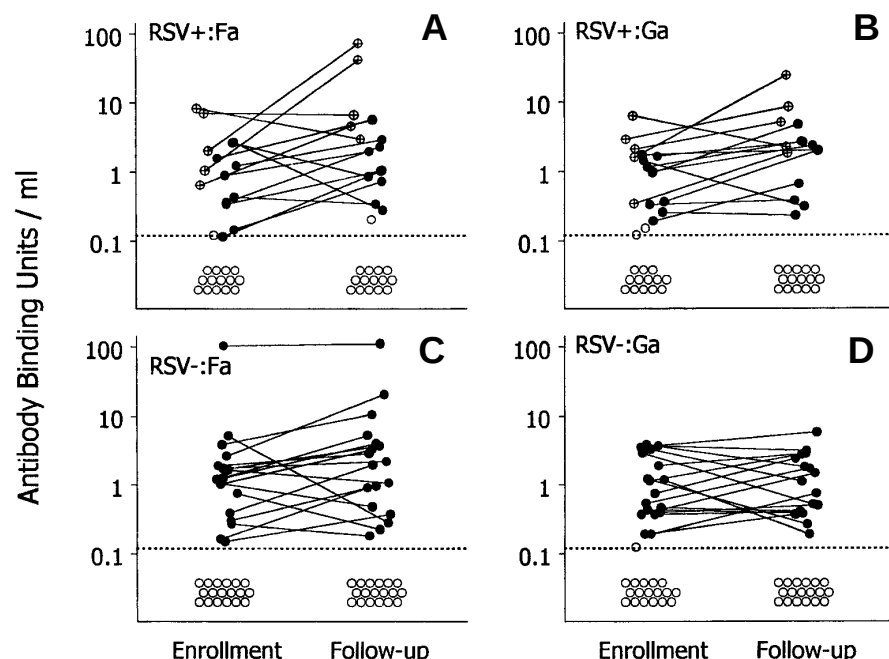


Fig 2. IgA and IgE antibody to RSV- F_a and G_a in nasal washes. Lines connect IgA values (solid circles) in nasal washes from the same patient obtained at enrollment and at follow-up. Levels of IgE antibody are indicated by open circles. **Panels A and B** show the results for IgA and IgE binding to RSV- F_a and G_a , respectively, in washes from RSV-positive patients ($n = 15$). **Panels C and D** show results for IgA and IgE antibody to RSV- F_a and G_a in washes from patients who tested negative ($n = 18$). Open circles with a cross represent IgA levels from 5 patients whose nasal washes were RSV-positive at both the enrollment and follow-up visits.

likely to be experiencing their first RSV infection and are more likely to have maternally acquired IgG antibody to RSV in their sera, as judged by their high titers of IgG antibody to the RSV- F_a and G_a antigens. Moreover, IgA IgG antibody to the RSV F and G glycoproteins has been shown in previous studies to be suppressed in the presence of maternally derived antibody during primary infection with RSV.^{25,31} Together, these findings suggest that IgE antibody to RSV may be difficult to detect at the time of a primary RSV infection during early infancy.

The clearest evidence to date that antigen-specific IgE can be detected in nasal secretions has come from studies of patients with sensitivity to pollen and dust mite allergens.³²⁻³⁴ In the 1960s, a clinical trial of infants who were sensitized to RSV with a formalin-inactivated vaccine raised the possibility that a subsequent infection

with this virus might be able to induce an eosinophil T_H2 lymphocyte response.^{35,36} No IgE antibody to RSV was measured at the time. In our study, IgE to RSV was not detected in nasal washes or sera from older infants (≥ 6 months old) who were more likely to have had a previous RSV infection. We speculate that antigens in the formalin-inactivated RSV vaccine might stimulate a different immune response or that RSV may be able to induce pulmonary eosinophilia without the production of antigen-specific IgE antibody.

Only one study has reported IgE antibody to RSV in sera from infants with wheezing.²⁹ However, the titers of IgE antibody measured were very low (<0.6% binding of added ^{125} I-labeled detecting antibody). We were not able to detect IgE antibody to RSV in the sera from our patient population. Welliver et al³⁷ were also not able to measure IgE antibody to RSV in sera

from their studies and reported that the production of IgE to RSV may therefore be a localized response in the airways. In their studies, the titers of IgE antibody to RSV were often highest in nasal secretions after the resolution of acute infection when the symptoms of wheezing had diminished or subsided.²⁰ The higher titers of IgE antibody to RSV that were measured, however, did not correlate with the persistence of wheezing or the development of positive skin test responses when the children were re-evaluated 7 to 8 years later.³⁸

Two previous studies have demonstrated the production of IgE antibody to the immunodominant RSV F and G antigens.^{21,30} In one study, 20 infants (<12 months of age) were examined (13 with bronchiolitis, 4 with pneumonia, and 3 with rhinitis). All infants were known to be experiencing primary infection with RSV. IgE responses were detected immediately and/or at follow-up in nasal secretions not only from 10 of the infants with bronchiolitis but also from 3 infants with pneumonia and from all 3 with rhinitis.²¹ In the other study, IgE antibody to RSV F and G (but not to 3 other, less immunogenic virion proteins from RSV) was detected in nasal secretions from infants with RSV bronchiolitis or pneumonia and in some of the infants with pneumonia who were not infected with RSV.³⁰ The mean age of the infants with bronchiolitis in that study was 4.3 months. There were no assessments of other antibody isotypes (IgA or IgG) for comparison. Neither investigation included asymptomatic control subjects.

In a more recent study, which also focused on infants who were <6 months of age, anti-RSV IgA antibody was demonstrated in nasal secretions obtained after hospitalization for RSV-induced wheezing.³⁹ However, no IgE antibody to RSV was detected. The patients in our study included infants with wheezing (60% of whom were hospitalized), infants with rhinitis, and control subjects without respiratory

tract symptoms. Older infants (aged 6 months to 2 years) were also evaluated. In our experiments we used an ELISA system and source of affinity-purified antigens similar to those used in the studies by Welliver et al.²¹ We also used a monoclonal anti-F_c E antibody on solid phase to capture IgE antibody in nasal wash supernatants, similar to the assay used by Russi et al.³⁰ Both IgA and IgG antibody isotypes to the 2 immunodominant RSV (F and G) antigens were easily detected in sera and nasal secretions from the majority of patients in our study, along with a boost in antibody titers at follow-up in samples from those who had positive test results for RSV at enrollment. However, we were unable to demonstrate the presence of IgE antibody to these antigens during or after an RSV infection.

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