

Association between pronounced IgA response in RSV bronchiolitis and development of allergic sensitization

Strannegård Örjan, Cello Jeronimo, Bjarnason Ragnar, Sigurbergsson Fridrik, Sigurs Nele. Association between pronounced IgA response in RSV bronchiolitis and development of allergic sensitization. *Pediatr Allergy Immunol* 1997; 8: 1–6. © Munksgaard 1997.

Forty-five children who had been hospitalized with bronchiolitis caused by respiratory syncytial virus (RSV) at a mean age of 4 months, and 90 matched control children, were tested for occurrence of RSV antibodies at one year of age. Of the children who had suffered from bronchiolitis, forty had demonstrable IgG antibodies, whereas the remaining five only had IgA antibodies against RSV. In the control group, 42% were RSV seropositive. The anti-RSV IgA antibody titres tended to be higher in patients with bronchiolitis than in controls and a larger proportion of the seropositive children in the former than in the latter group had demonstrable IgG antibodies. These findings suggest that RSV infections causing bronchiolitis are more often associated with a strong antibody response than are mild cases of the infection.

Follow-up of the children at 3 years of age showed that allergic sensitization and development of asthma had occurred much more frequently in children with past RSV bronchiolitis than in controls. Children with past RSV bronchiolitis who later developed allergic sensitization had elevated RSV IgA antibody titres at one year of age more frequently than children with past RSV-bronchiolitis, who were not sensitized ($p=0.015$). No significant differences regarding IgG antibody titres were observed. Since IgA, similarly as IgE, antibody formation is strongly Th2 cell dependent, the results are compatible with other findings suggesting that RSV has an unusual propensity to activate the Th2 cell system. This may contribute to the pathological picture of bronchiolitis in small children and at the same time render the infected child predisposed for later development of allergic sensitization. RSV bronchiolitis may thus be an important risk factor for later development of atopic disease although it cannot be excluded that the bronchiolitis simply serves as a marker that predicts later development of atopy.

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Key words: RSV; bronchiolitis; IgA response; atopy

Received June 3, 1996
Accepted January 14, 1997

In a previously reported prospective study of infants who had been hospitalized with RSV induced bronchiolitis, and their matched controls, the results obtained indicated that RSV bronchiolitis during the first year of life is an important risk factor for the development of asthma during the subsequent two years (1). Thus, 22% of 47 children hospitalized with RSV bronchiolitis developed asthma compared to 1% of the 94 controls. RSV bronchiolitis was also associated with increased frequency of allergic sensitization, as serum IgE antibodies to

common allergens were seen in 32% in the bronchiolitis group compared to 8% in the control group. RSV was an independent risk factor for asthma and sensitization but the combination of RSV bronchiolitis and heredity for asthma and atopy further increased the risk. The frequency of asthma or sensitization was not increased among controls who had experienced a mild or subclinical RSV infection, indicating that only bronchiolitis severe enough to cause hospitalization is a risk factor for later atopy/asthma. These results suggested that RSV may be a

trigger factor for atopy in childhood, although, as pointed out by Welliver, a predisposition to immediate hypersensitivity could have preceded as well as provoked episodes of both RSV bronchiolitis and asthma (2).

Since RSV bronchiolitis in the previous report by Sigurs and coworkers (1) was associated not only with increased frequency of asthma but also with increased frequency of allergic sensitization to common allergens, it appears possible that RSV, through mechanisms influencing the immune system, may trigger the development of atopic disease. With regard to the possible immunomodulatory effects of RSV, Welliver and coworkers earlier demonstrated specific anti-RSV IgE antibodies in nasal secretions of infants with bronchiolitis, and also found a relationship between IgE antibody titres in the secretions and the frequency of later appearing attacks of wheezing bronchitis (3, 4, 5). On the basis of these results it was suggested that RSV, via induction of anti-RSV IgE antibodies, may give rise to immediate hypersensitivity reactions leading to bronchiolitis and, upon repeated infections, to episodes of wheezing bronchitis.

Based on the results of animal experiments showing that parainfluenza or distemper viruses may have a general adjuvant effect on allergic sensitization (6), an alternative explanation for the results of Welliver et al. may be offered. This explanation would be that RSV, which in experiments in mice has been found to have an unusual ability to activate Th2 cells (7–9), may shift the balance between Th1 and Th2 cells (10) towards the Th2 side, thereby making the affected individual prone to develop allergic sensitization to various allergens. In preliminary experiments involving *in vitro* stimulation of human peripheral blood cells we recently found that RSV had a greater ability than enteroviruses to induce production of IL-4, which is a Th2 cytokine, whereas the reverse was true regarding the Th1 cytokine IFN- γ (Strannegård et al., unpublished). Based on these findings and those obtained in animal experiments, we considered that it would be of interest to characterize the serum antibody response that follows RSV-induced bronchiolitis. Similarly, as is the case with IgE antibody, the formation of IgA antibodies is Th2 cell dependent, being particularly sensitive to the stimulatory effect of IL-10 (11, 12). Therefore, if RSV has indeed an unusual ability to activate Th2 cells, one would expect not only production of IgE antibodies (3, 4, 5) but also a pronounced production of IgA antibodies to occur following RSV-induced bronchiolitis. In the present study we provide evidence that there is indeed a strong serum anti-RSV IgA response in RSV-induced bronchiolitis and that a pronounced response is associated with a later appearance of allergic sensitization.

Materials and methods

Patients and controls

The infants with bronchiolitis and the controls were the same as those described in a previous publication (1). Briefly, the parents of all infants hospitalized with laboratory-verified RSV in the pediatric ward, Borås Central Hospital, Sweden, from December 1989 to April 1990, were invited to take part in the study. A diagnosis of RSV infection was obtained by demonstration of RSV antigen in nasopharyngeal secretions by a rapid enzyme immunoassay (Testpack RSV, Abbott Laboratories). Out of a total of 52 infants with RSV bronchiolitis 47 were followed up at the mean ages of 1 year and 3 years.

For each child with RSV bronchiolitis, the parents of the two children with birth dates closest to the index children and of the same sex and from the same local Child Health Center were contacted and asked to participate in the study. Only three families refused to participate but two of the infants could be exchanged for another two infants with birth dates that were also close to those of the RSV infected children. Thus 93 control children participated in the study.

As previously reported in detail (1) the characteristics of the two groups of children were remarkably similar except for a slight difference regarding mean duration of exclusive breast feeding (RSV group 2.4 months, control group 3.1 months). Thus, double heredity for atopy was observed in 19% in both groups, single heredity in 38% and 37% for the RSV group and the controls, respectively, and double heredity for asthma in 0% and 3%, respectively. Similarly, only minor differences between the two groups were observed regarding mean gestational age, mean birth weight, mean weight at second follow-up and number of siblings in the family. One of the control children had been hospitalized with RSV-negative pneumonia at the age of 6 weeks, but otherwise none had been hospitalized for respiratory symptoms between the neonatal period and the first examination.

All children underwent two follow-up examinations, the first one at a mean age of 1 year and the second at an average age of 3 years. At the first follow-up serum samples were obtained and tested for anti-RSV antibodies and at the second follow-up sera were tested for IgE antibodies against common allergens using Fx5[®] and Phadiatop[®] (screening tests for common food and inhalant allergens respectively, Pharmacia Diagnostics, Uppsala, Sweden).

Ethics

All parents of the participating children gave their oral consent after receiving oral and written infor-

Table 1. Anti-RSV IgG and IgA antibodies in one year old children with or without past RSV-bronchiolitis

Bronchiolitis	IgA-antibodies	IgG antibodies		Total
		Present	Absent	
Yes	+	22	5	27
Yes	-	18	0	18
No	+	8	14	22
No	-	16	52	68

mation. The study was approved by the Human Research Committee of the Medical Faculty of Gothenburg University.

RSV IgA and IgG antibody determination

An RSV-specific indirect enzyme linked immunosorbent assay (ELISA) was used for the detection of IgA and IgG responses in serum samples. The RSV antigen was prepared as described previously (13). Polystyrene microtitre plates (Maxisorp; Nunc AS, Roskilde, Denmark) were coated with the viral antigen diluted to a concentration of 10 µg/ml in carbonate buffer, pH 9.6, and incubated overnight at 4°C. Remaining binding sites in the wells were blocked for 1 hour at 37°C with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Serum samples were diluted serially on the plates in twofold dilution steps (starting dilution 1/100) in PBS containing 1% BSA and 0.05% Tween and incubated for 2 hours at 37°C. After washing, alkaline phosphate-conjugated goat-anti-human IgA or IgG (Jackson Laboratory, Bar Harbor, ME), was added and allowed to react for 90 min at 37°C. Following several washes, the plates were incubated with the substrate (p-nitrophenyl phosphate dissolved in diethanolamine buffer, pH 9.8) and read at 405 nm. The specificities and sensitivities of the two tests were established using a variety of RSV-positive and negative reference sera including sera from newborns. These tests showed that there was no crossreactivity between IgG and IgA antibodies and that non-specific reactions occasionally occurred at serum dilutions of 1/50 but not at dilutions equal to or exceeding 1/100. The intra- and interassay coefficients of variation were consistently below 15%.

From 45 of the 47 children with past RSV bronchiolitis and from 90 of 93 controls serum samples were available for both IgG and IgA tests. Serum from an additional child with bronchiolitis was tested for IgA antibodies only.

Statistics

Group comparisons of antibody titre values were performed with Mann-Whitney U-test and comparisons of proportions using Fisher's exact test.

Results

Antibody prevalences and titres in controls and in children with past RSV bronchiolitis

In the control group 24 of 90 (27%) tested children were positive for IgG antibodies and an additional 14 (16%) were positive for IgA but not for IgG antibodies, giving a total seropositivity rate of 42% (Table 1).

Forty (89%) of 45 sera in the RSV bronchiolitis group were positive for IgG antibodies and the remaining five all positive for IgA antibodies, together giving a seropositivity rate of 100% (Table 1). Serum from an additional child that was tested for IgA only, was also positive. As seen in Table 1, a higher proportion of the seropositive cases among the control children than among the children with past bronchiolitis showed evidence of past RSV infection solely in the form of demonstrable anti RSV IgA antibodies (14/38 vs. 5/45; Fisher's exact test, $p < 0.01$). Thus, RSV infections leading to bronchiolitis were more frequently associated with a detectable IgG response than were less severe RSV infections.

Although seropositivity rates differed between controls and cases with RSV-bronchiolitis, the actual IgG antibody titres were similar. By contrast, IgA antibody titres were slightly but not significantly higher in the group with RSV bronchiolitis than in the control group (Mann-Whitney U test, $p = 0.13$). The latter group generally displayed only low titres of IgA-antibodies. Thus, moderate to high antibody titres (i.e. ≥ 200) were noted in two of the 22 IgA-positive controls (9%) whereas the corresponding ratio for the cases with bronchiolitis was 9/27 (33%) (difference not significant; Fisher's exact test, $p = 0.08$).

Antibody titres in children with past RSV bronchiolitis in relation to later development of allergic sensitization and asthma

In the RSV bronchiolitis group of 47 children, 14 children had developed specific IgE antibodies at follow-up at 3 years of age, as revealed by positive reactions to one or both of the allergen mixtures employed, and 11 children had developed asthma (five of these were also sensitized). By contrast, among the 93 controls, seven children were sensitized and only one child had asthma.

In the RSV bronchiolitis group, the sensitized children had slightly, but not significantly higher

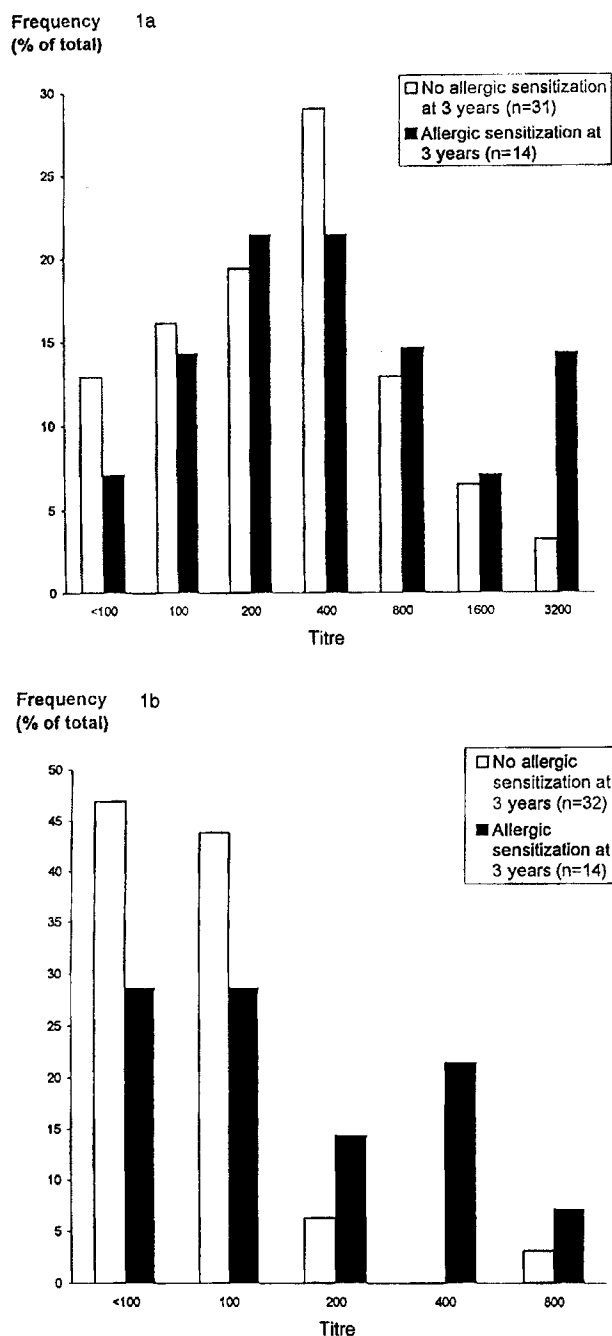


Fig. 1. Frequency distribution of anti-RSV IgG (1a) and IgA (1b) antibody titres in one year-old children with previous, confirmed RSV-bronchiolitis in relation to later allergic sensitization.

anti RSV IgG antibody titres at one year of age when compared to non-sensitized children (Fig. 1a). Similarly, the IgA antibody titres were higher in the children developing sensitization than in those not sensitized (Fig. 1b). The frequency of clearly elevated IgA antibody titres, defined as titres exceeding median values of IgA positive controls, was signifi-

cantly higher in sensitized (43%) than in non sensitized (9%) children (Fisher's exact test, $p=0.015$, Table 2). The frequency of clearly elevated IgA titres was also high in children who developed asthma (four of 11). This, however, appeared to be due to an association between asthma and sensitization, since three of these four children also had specific IgE antibodies and asthma was not associated with increased frequency of elevated RSV IgA antibodies in the absence of allergic sensitization. Regarding RSV IgG antibodies, no significant differences between elevated antibody levels in the different groups were observed (Table 2).

Among six (out of seven) sensitized children in the control group tested for IgA and IgG antibodies two had IgA antibodies with titres 100, and another one had IgG antibodies, titre 100. The one child with asthma had an IgA titre of 100. Thus, only low antibody titres were seen in these children.

Discussion

The antibody response during RSV infection is characterized by a transient IgM response, an IgA response that lasts for several weeks to months and a sustained IgG response (3, 14). In RSV bronchiolitis, which occurs with peaking incidence at the age of 2–7 months (15, 16), a serological diagnosis cannot always be obtained even if antibodies belonging to the IgG, IgA as well as IgM class are being assayed (17). Antibody titres resulting from RSV infection in 1–8 month old infants have been found to be 15% to 25% of those of older subjects (reviewed in ref no. 18). In particular, a rise of IgG anti RSV-antibodies is frequently not demonstrable (3, 14, 17, 19), probably due to immaturity of the immune system or suppression of the IgG response by maternally transferred antibodies (20). In view of these findings it is not surprising that not all cases with a confirmed RSV-infection had demonstrable IgG antibodies in the present study.

Although a larger proportion of seropositive cases in the bronchiolitis than in the control group had demonstrable IgG antibodies the actual titres of these antibodies were similar. By contrast, IgA antibody titres tended to be higher; thus IgA antibody titres above median values were found in 33% of the bronchiolitis cases but in only 9% of the controls. This would suggest that RSV bronchiolitis had been associated with a more vigorous IgA response than had milder RSV infection. A strong IgA response would be supposed to occur after unusually abundant release of the cytokines TGF- β , which is a switch factor for IgA synthesis and/or production of the Th2 cell cytokines IL-10, IL-6 and IL-5, which all have strong stimulatory effects on IgA synthesis (11, 12).

Table 2. Frequency of elevated anti-RSV antibody titres in children with past RSV-bronchiolitis

Category of children	No. with elevated titres*/total no. of cases (%)	
	IgA	IgG
Sensitized	6/14 (43)**	5/14 (36)
Not sensitized	3/32 (9)	7/31 (23)
Asthma	4/11 (36)	3/11 (27)
Asthma, but not sensitized	1/6 (17)	0/6 (0)
Not sensitized, no asthma	2/26 (8)	7/25 (28)

* Elevated titres were defined as titres exceeding median of positive controls (i.e. for IgA ≥ 200 and for IgG ≥ 800).

** Differences between sensitized and non-sensitized children as well as between sensitized children and non-sensitized without asthma are statistically significant (Fisher's exact test, $p=0.015$ and $p=0.014$, respectively).

The possibility that a pronounced IgA synthesis in RSV infection may be secondary to a preferential stimulation of Th2 cells by RSV gets support from experimental studies in mice. Thus, Openshaw and coworkers have shown that a protein of the RSV envelope, i.e. the heavily glycosylated glycoprotein G, has an unusual ability to activate Th2 type CD4⁺ cells (8, 9). Furthermore, transfer of Th2 cells from RSV G protein-immunized mice followed by RSV challenge resulted in pneumonia associated with heavy eosinophilia in the mice. In preliminary experiments we have found that RSV is a better inducer of IL-4 and a less potent inducer of IFN- γ than are enteroviruses (Strannegård et al., to be published). Thus, the present results would be compatible with the suggestion that RSV infection results in Th2-cell mediated inflammation, associated with a pronounced IgA response and presumably also an IgE response. Neither IgA nor IgE-antibodies may, however, necessarily be directly involved in the pathogenesis of the inflammatory reaction. In this context it should be noted that Welliver and coworkers (3, 4, 5) and later Bui et al. (21) have found increased levels of specific IgE anti-RSV antibodies in nasal secretions and serum, respectively, although others have failed to confirm these results (22).

Judging from our present results, earlier demonstrations of IgE responses associated with RSV infections, and the experimental data obtained in mice, an early RSV infection in infancy associated with bronchiolitis might be supposed to predispose for development of atopy. In fact, the previous results of Sigurs et al. (1) agree with this assumption. Thus in this study by the far strongest risk factor for development of asthma at 3 years of age was previous RSV bronchiolitis. In fact, in the absence of previous RSV bronchiolitis, a family history of atopy was not associated with development of asthma in any child, whereas the combined occurrence of heredity and previous RSV bronchiolitis was associated with asthma in 55% of the children. Similarly,

RSV bronchiolitis seemed to be a more important risk factor for allergic sensitization than heredity for atopic disease. The combination of both factors was associated with sensitization in one third of the children.

Although a causal relationship between early RSV infection and later development of allergy is not established (2) it seems that the findings described above make such a relationship probable. In the present study we obtained additional evidence for this hypothesis. Thus, there was a significant association between a strong anti-RSV IgA response and later development of allergic sensitization, suggesting that severe RSV infection in early infancy predisposes for later development of atopic allergy. The reverse possibility, i.e. that an atopic constitution predisposes for increased severity of RSV infection, seems less likely, since there was almost exactly the same level of heredity for atopic disease in the children with bronchiolitis as in the controls. Similar results were obtained in another study of children with RSV bronchiolitis and controls, which also failed to demonstrate any difference regarding family history of atopy in the two groups (23). These findings do not, however, exclude the possibility that severe RSV infection, i.e. bronchiolitis, simply serves as a marker that predicts later development of atopic disease.

In summary, the present results give further support to previously made claims that RSV has an unusual ability to activate Th2 cells (7, 8) probably due to the occurrence of a particular glycoprotein (G) on the surface of the virus (9). The sequence of events leading to bronchiolitis in small children may then be the following: During RSV infection Th2 cells are activated leading to formation of several cytokines, among others IL-4, IL-5 and IL-10. IL-4 stimulates IgE synthesis and IL-10 enhances IgA synthesis but none of these immunoglobulins may necessarily have any impact on the immediate pathogenesis of the disease. The production of IL-5 will lead to accumulation and activation of eosinophils and subsequent release of highly toxic eosinophilic proteins (24, 25, 26). Together with increased release of histamine and leukotrienes (5, 27) as a result of direct effects of the virus (28) or possibly indirectly, via effects of RSV-specific IgE antibodies (3), bronchial obstruction will ensue. The reason why some children subsequently develop RSV bronchiolitis and some only milder forms of RSV infections is not clear, however. An important prerequisite for development of bronchiolitis might be diminished lung function prior to the infection (29, 30).

Since the Th2 cell immune system is subject to autostimulation by the secreted cytokines, in particular IL-4 and IL-10 (10), it seems possible that a

strong stimulation, such as that which may be associated with RSV infection at an early age, may give rise to long-lasting effects leading to predisposition for allergic sensitization. The present results are entirely compatible with this hypothesis and suggest that RSV bronchiolitis is a trigger factor for allergic sensitization and later development of atopic disease.

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