

Salivary anti-RSV IgA antibodies and respiratory infections during the first year of life in atopic and non-atopic infants

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Salivary SIgA antibodies against RS virus were studied in 105 children during the first year of life. The infants were divided into groups according to their risk of atopy. At birth 13 neonates showed measurable amounts of SIgA to RS virus. In another 26 children specific antibodies were detected but in concentrations too low for quantitative analysis. During the first year of life this increased to 29 antibody-positive samples with measurable amounts of antibody and 39 with concentrations too low for quantitative determination. At this time 8 children of the high risk group had developed symptoms of allergy. None of these children had measurable amounts of SIgA anti-RSV in their saliva. In comparison, 10 of the remaining 26 high risk infants without symptoms of allergy did have such antibodies. Atopic infants had significantly more respiratory infections during the first year of life than nonatopic infants. The avidity of SIgA anti-RSV in neonatal samples was significantly higher than avidity determined in breast milk SIgA but comparable to the avidity of serum IgG. During the first year of life a continuing decrease of salivary SIgA avidity was observed.

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The concentration of total SIgA in the saliva of newborns is minimal and was found below the limit of detection in most reported studies. However, specific SIgA to *E. coli*, poliovirus (1), and milk proteins (2) has been detected as early as the first day of life. As IgA does not pass the placenta these antibodies must be of fetal origin and their concentrations should reflect regulatory mechanisms related to the newborn's immune system. In accordance with this concept, Renz et al. found a correlation between elevated concentrations of SIgA against casein in the newborn and the subsequent development of atopic disease (3, 4).

As viral infections are more frequent in allergic children than in their non atopic siblings and on the other hand have been linked to the onset of manifest allergies during the first year of life, active immunity against viral antigens, in particular respiratory syncytial virus, in the neonate appears of particular interest (5–7).

It was the purpose of the present study to investigate the development of SIgA anti-RSV

during the first year of life and to correlate this parameter to atopic disease.

Material and methods

Study group

We studied 105 children (46 girls, 59 boys), consecutively born after uneventful pregnancies and deliveries at the Department of Obstetrics and Gynaecology, University of Marburg, between December 1988 and August 1989. Parents had to give informed written consent before their infants were included in the study. The study was approved by the Ethics' Committee of the medical faculty.

Infants were divided into groups according to their risk of developing atopy. A high risk group (Group 1, $n = 34$) for allergies was defined by a cord blood IgE > 0.9 IU/ml and/or manifest atopic disease in both parents. A cord blood IgE of 0.9 IU/ml was chosen as a cut off as this value had been found to be optimal by previous large studies on allergy prediction (8, 9). A risk group (Group

2, $n = 43$) was defined by a normal cord blood IgE ≤ 0.9 IU/ml and manifest atopic disease in one of the parents. The control group (Group 3, $n = 28$) had CB-IgE < 0.09 IU/ml, parents without a history of atopic disease and without allergen-specific serum-IgE. The groups were comparable with respect to sex, social background, parental smoking, and the proportion of breastfeeding. Each infant was seen at birth and three more times during the first year, when a history was taken and a complete physical examination was performed. In addition, parents were advised to report respiratory infections immediately and to bring their children for rapid viral antigen detection if possible.

Sample collection

Unstimulated saliva samples were obtained over 20–30 minutes, using a saliva absorbant cellulose swab. Collection was carried out within 8–72 h after birth and at nominal sampling times of 3, 6, and 12 months at least 2 h after the last meal. We took samples as closely as possible to the nominal sampling times. Actual collection times were approximately symmetrically distributed about these nominal times.

Samples were heated for 20 min at 56°C, centrifuged at 5000 rpm for 15 min, and stored at -70°C until being tested. During the first week after delivery breast milk was collected in sterile covered glass jars from 87 mothers. Immediately after collection, samples were centrifuged at 9000 rpm for 15 min at 4°C to remove fat and cellular elements. The milk samples were then divided into aliquots and stored at -70°C until being tested. Venous blood was obtained from 99 mothers following delivery. Umbilical cord blood was obtained in all infants after the cord had been wiped of maternal blood in order to avoid contamination. All blood samples were allowed to clot at room temperature. Samples were then centrifuged, and serum was stored at -20°C until analyzed.

Determination of SIgA anti-RSV

Preparation of RS virus was performed according to Sarkkinen (10). Microtiter plates (Nunc laboratories, Wiesbaden, FRG) were coated with antigen and with control antigen consisting of noninfected cell lysates. A direct ELISA technique was used for SIgA determination. Saliva and breast milk samples were serially diluted in POD buffer (Behringwerke, Marburg, FRG) beginning with 1:10, and 100 μl of diluted sample was added to the antigen-coated wells and incubated at 37°C for 1 h. After washing with 0.05% Tween in normal saline peroxidase-conjugated goat anti-human secretory

IgA (α -chain and secretory component specific, Bio-Science Products, Emmenbrücke, Switzerland) was added. The enzymatic reaction with the substrate TMB (Tetramethylbenzidine, Behringwerke, Marburg, FRG) was stopped by adding 1 M H_2SO_4 . The photometric measurement was performed at A_{450} in a microplate autoreader (Titertek Multiscan, Flow Laboratories, Meckenheim, FRG). ELISA units were expressed as a reciprocal value of the dilution which yielded an OD of 0.5. Samples were always run in duplicates.

The following control incubations were carried out: incubation of control antigen with children's saliva gave always an OD below 0.150. Incubation of antigen with saliva of a patient with IgA deficiency gave always an OD below 0.075.

Determination of IgG antibodies against RS virus in sera

IgG anti-RSV in maternal sera were determined by a direct ELISA technique. Microtiter plates were coated with RS virus antigen, serial dilutions of the sera were added and the plates were incubated for 1 h at 37°C . After washing with 0.05% Tween in normal saline, peroxidase-conjugated rabbit anti human IgG (fc-specific, Behringwerke, Marburg, FRG) was added, and the plates were incubated for another hour. The enzymatic reaction with the substrate TMB (Behringwerke, Marburg, FRG) was terminated by adding 1 M H_2SO_4 . The photometric measurement was performed according to the system described for SIgA determination but read at an OD of 1.0. Control antigen consisted of non-infected cell lysates which were treated in the same way as the infected cell lysates.

Measurement of total serum IgE

Total serum IgE of cord blood and maternal blood was measured by Enzygnost® IgE ELISA kit (Behringwerke, Marburg, FRG). Parents' specific IgE was measured by Phadiatop®. The tests were carried out according to the manufacturer's instructions.

Determination of antibody avidity

We used an ELISA method based on the ability of urea to interfere with the binding of low avidity antibodies to the antigen by hydrogen bonds disruption (11). Quadruplicate assays of SIgA and IgG determinations were performed as described above. Following incubation of the samples, one duplicate was washed with 0.05% Tween in normal saline containing 8 M urea while the other duplicate was washed with 0.05% Tween in normal saline only. The assays were finished in the usual way

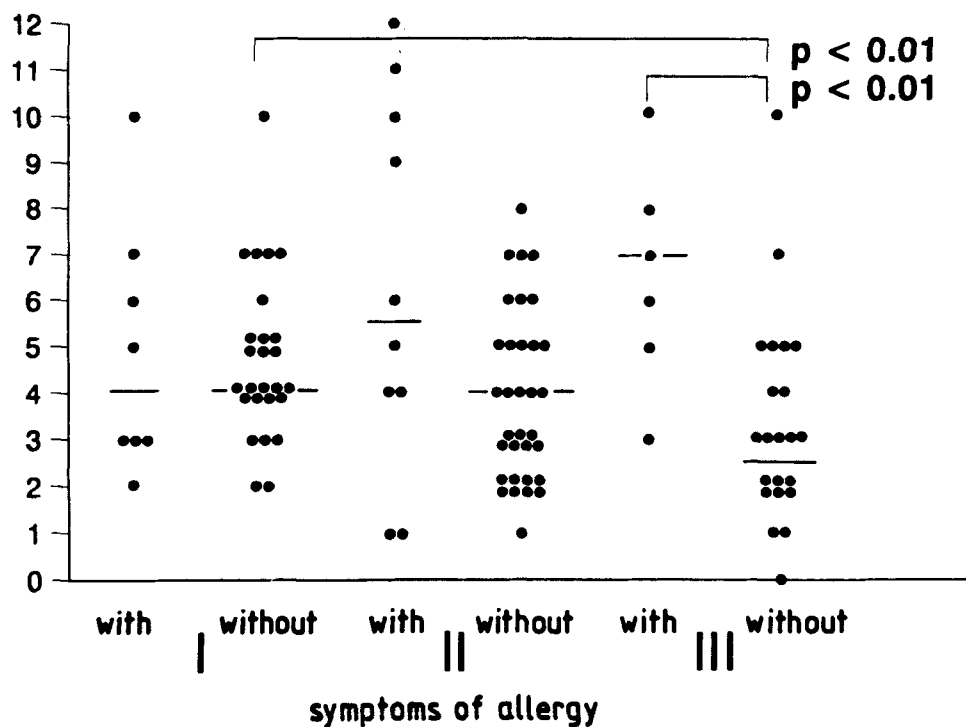


Fig. 1. Number of respiratory infections in infants of Groups 1 (high risk), 2 (risk) and 3 (control) with and without symptoms of allergy during the first year of life. The number of upper respiratory infections is given on the vertical axis.

and ELISA units were calculated for both assays. The ratio of the two values (ELISA units with urea divided by ELISA units without urea) gives a reflection of antibody avidity. The closer the end point ratio was to 1.0 the higher the avidity of the antibody.

Rapid detection of viral antigen

Nasal secretions were obtained by suction with a soft tube and immediately assessed by direct immunofluorescence using a mouse anti-RSV-monoclonal antibody (Whittaker Bioproducts, Walkersville, MD, USA).

Statistical analysis

Groups were compared using the Mann-Whitney U-test and Fisher's exact test.

Results

Study population

The risk of newborns to develop atopic disease was judged by the parents' allergy history, the parents' total and specific serum IgE, and by determination of cord blood IgE. By these criteria, 34 newborns were assigned to the high risk Group 1. Fifteen of these infants had two parents with manifest atopic disease, one of these also had an elevated cord blood IgE > 0.9 IU/ml. Another 19 infants had elevated cord blood IgE levels between 0.91 and 4.1 IU/ml, but no detectable cord blood IgA ruling

out significant contamination of cord blood by maternal blood. In 17 of these infants one parent had manifest atopic disease, only 2 infants had parents with no history of atopic disease and normal serum IgE. A total of 43 infants were in the risk Group 2 with one allergic parent but normal cord blood IgE.

The prevalence of bronchial asthma and allergic rhinitis in fathers and mothers was nearly the same. Atopic dermatitis in fathers was only half as frequent as in mothers.

Control Group 3 consisted of 28 infants whose parents had no history of atopic disease, the parents' total serum IgE, specific serum IgE and the infants' cord blood IgE were normal.

Clinical course

The study took part between December 1989 and August 1990 so that two winter seasons were included none of which, however, brought an RSV-epidemic to Marburg.

All infants were examined at 3, 6, and 12 months of age by one of the investigators who had no knowledge of the infants' immunological parameters at birth. The following symptoms and signs were recorded as manifestations of allergic disease: atopic dermatitis of at least 6 weeks duration, wheezing with cough of at least 4 weeks duration, vomiting, colic, diarrhea, or urticarial reactions in connection with feeding which cleared after elimination of the incriminated food, recurrent

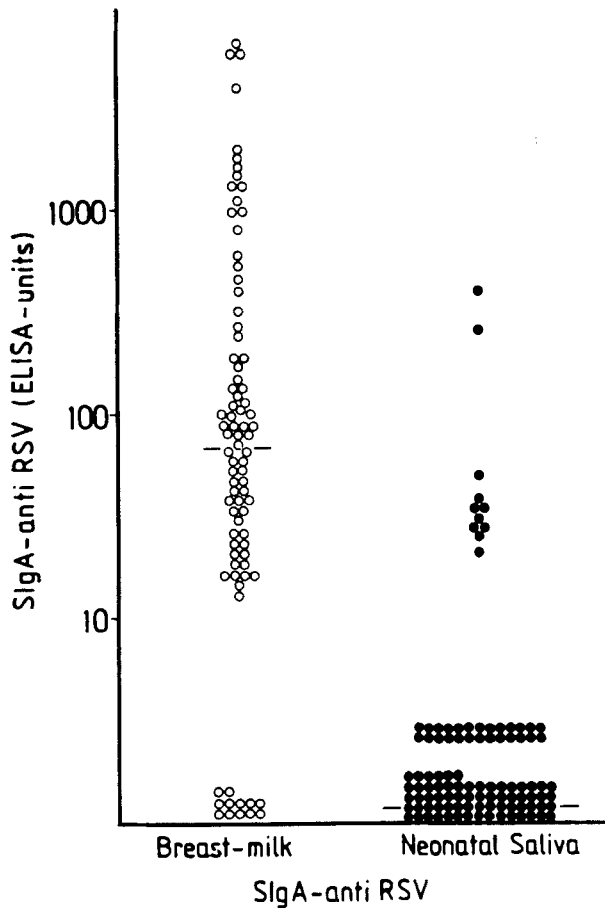


Fig. 2. SIgA-Antibodies against RSV in breast milk and in neonatal saliva.

sneezing and clear or white nasal discharge of at least 6 weeks duration.

At the same time symptoms and signs of upper respiratory infections were recorded such as cough and nasal discharge of a few days duration or intercurrent febrile episodes.

At one year of age 24 children (23%) exhibited allergic disease according to our definition. Fourteen children showed atopic dermatitis, 5 children

asthma, 4 children had a combination of both, and one child an urticarial reaction after ingestion of cow's milk. Surprisingly, there was no difference in the prevalence of allergy between the risk groups during the period of observation.

Few parents complied with our request to present their infants early during a course of infection. Therefore we could obtain only 18 samples of nasal secretions for rapid detection of viral antigen. In these infants, no RSV infection was identified.

At the scheduled visits we asked for respiratory tract infections. During the first year of life 0–12 (median 4) episodes of URI were reported. There were significant differences between the risk groups and between infants with and without symptoms of allergy (Fig. 1). In the high risk Group 1 infants with and without symptoms of allergy had 4 (median) episodes of URI. In the control Group 3 atopic infants had 6.5 (median) episodes of URI while infants without symptoms of allergy had 2.5 (median) episodes of URI. The risk Group 2 fits in between: infants with allergy had 5.5 (median), infants without allergy 4 (median) episodes of URI. Since at least 80% of infants in Group 1 and 50–60% in Group 2 will eventually develop a manifest allergy infants of Group 3 without allergy may be regarded as the relevant non-atopic control group. These infants had significantly fewer episodes of URI than the rest of the children ($p < 0.001$).

Local antibodies to RS virus in infants

Measurable amounts of secretory IgA antibodies to RS virus were found in 11 infants at birth. In another 26 neonates specific antibodies were detected but in concentrations too low for quantitative analysis (Fig. 2). There was no correlation between the concentration of salivary antibodies and the time of birth so that no conclusion seems

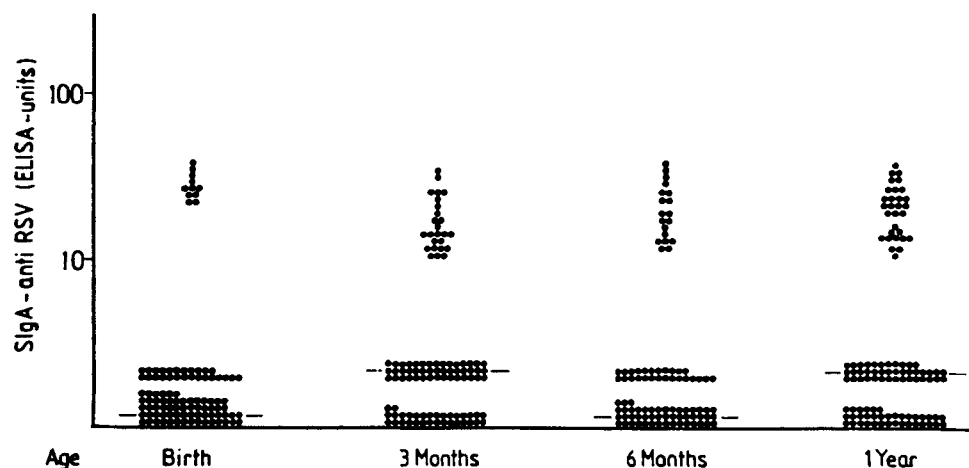


Fig. 3. SIgA-Antibodies against RSV in saliva during the first year of life.

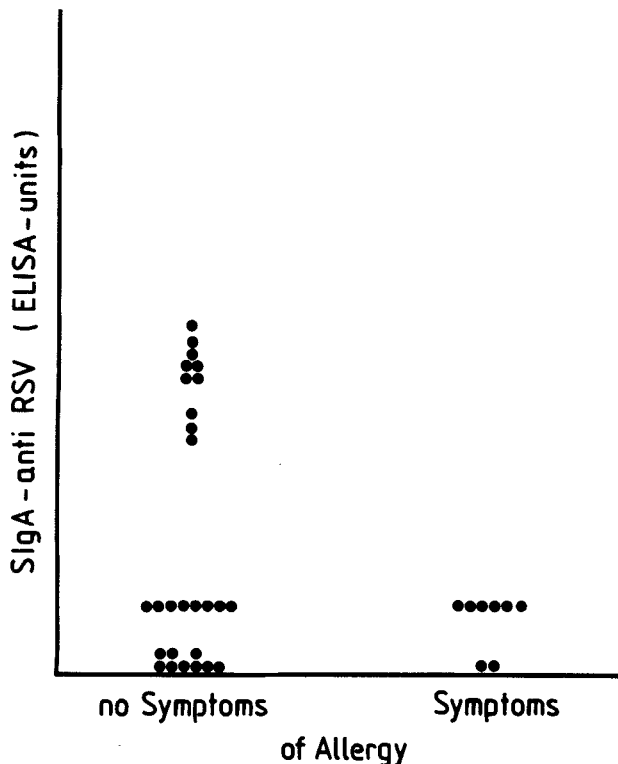


Fig. 4. SIgA-Antibodies against RSV in saliva of high risk infants with or without symptoms of allergy at 1 year of age.

possible as to a maternal RSV-infection as a possible factor influencing the height of the infant's antibody response.

Determination of salivary SIgA anti-RSV was repeated at 3, 6, and 12 months of age. During this time the number of antibody-positive samples increased, both with measurable amounts of antibody and with concentrations too low for quantitative determination. At one year of age 30 children showed measurable amounts of antibody, 41 children had antibody concentrations which were not

measurable. The median of the group was zero at birth and did not change during the first year of life (Fig. 3). The same holds true for the allergy risk subgroups.

At the end of the first year of life 8 children of the high risk group had developed manifest symptoms of allergy. None of these children had measurable amounts of SIgA anti-RSV in their saliva while 10 of the remaining 26 Group 1 children did have such antibodies (Fig. 4). This difference was significant ($p < 0.05$, Fisher's exact test).

Antibodies to RS virus in mothers

Since the mechanism of SIgA formation in the neonate is still not clear, the immune status of the mother was investigated. Circulating IgG antibodies to RSV were detected in 97 of 99 (98%) maternal sera. Concentrations of circulating IgG anti-RSV did not differ between women with manifest allergy and non-atopic women. In breast milk, 77 out of 87 (89%) samples showed SIgA anti-RSV (Fig. 2). There was no difference between atopic and non-atopic women.

Antibody avidity

For better characterization of the neonatal SIgA antibodies we determined antibody avidity. In only five neonates antibody concentrations and the amount of saliva available allowed this determination. In these infants the avidity of salivary SIgA anti-RSV was high with a median of 0.81 (0.67–1.00). During the first year of life the salivary SIgA anti-RSV avidity decreased significantly ($p < 0.02$) with a median of 0.0 (0.0–0.75) at one year of age. Breast milk SIgA anti-RSV avidity had a median of 0.37 (0.02–0.64) while serum IgG anti-RSV avidity was 0.85 (0.4–1.0). Thus, neonatal salivary

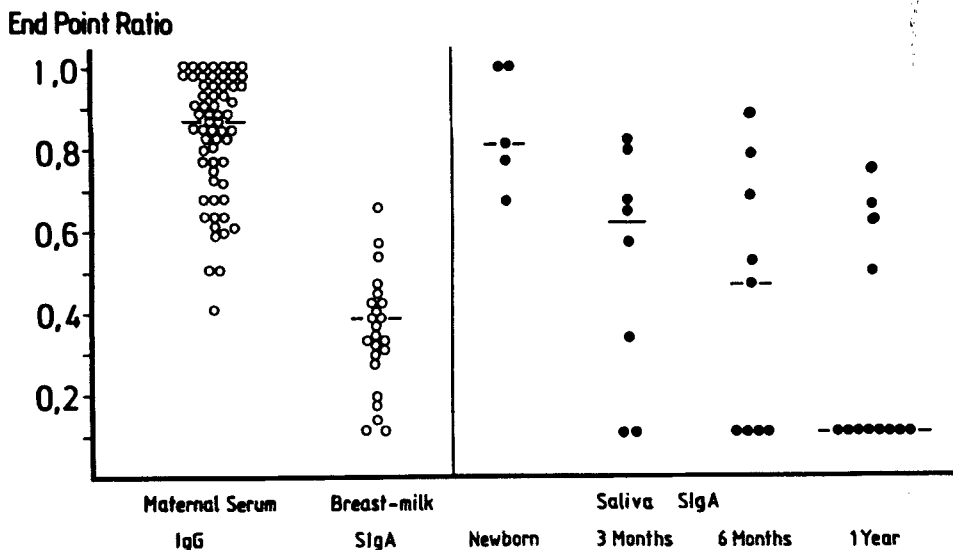


Fig. 5. Avidity of antibodies against RSV in maternal serum, breast milk and in the infants' saliva during the first year of life.

SIgA anti-RSV avidity was comparable to that of serum IgG, but significantly ($p < 0.001$) higher than the avidity of breast milk SIgA anti-RSV (Fig. 5).

Discussion

The concept of secretory immunity in infancy and childhood has changed during recent years. It is clear now that specific SIgA is actively secreted by the newborn. Since the first demonstration (1) this has been confirmed for different kinds of antigens as *E. coli*, poliovirus (1), and cow's milk proteins (2). We were interested in secretory immunity to RS virus since this virus accounts for a high morbidity in infants. Not only is RS virus the major cause of hospitalization in infants in the Western world (12) but following bronchiolitis a high percentage of children experience subsequent episodes of wheezing (13–15) and some surveys suggest a link between bronchiolitis and atopy (6, 7), although the exact relationship between RSV infection and atopy is still unclear.

Production of serum antibody to RSV is relatively deficient in infants younger than 6 months of age at the time of infection (16). Also, titers of RSV serum antibody decline rapidly after primary infection while more persistent antibody responses occur after reinfection (17). The secretory response to RSV infection has its peak titers 14–28 days after infection and declines sharply at 90 days after primary infection (18).

There is still little known about the development of secretory antibody titers to RSV independently of an acute RSV infection (19). We were unable to demonstrate RSV infections in our study despite a presumed infection rate of 60% in the first year of life. This may well have been due to the fact that parents did not present infants early during a course of infection or not at all. In fact, at the scheduled visits careful assessment of the interim histories revealed many episodes of upper respiratory infections which had not been reported before. Our allergic infants had more infections during the first year of life than non allergic infants. To our knowledge this has only been reported once before (20). It is unlikely that respiratory symptoms of atopy were mistaken for infections since most infants with manifest atopy had skin symptoms.

SIgA anti-RSV titers showed little change during this year and antibody avidity even decreased. This can be explained either by a lack of exposure which seems rather unlikely in view of other authors' experience or may indicate the need for recurrent infections in order to boost "congenital" antibody. These findings remind of the SIgA-re-

sponse to milk proteins which also shows a rapid decrease during the first months of life in spite of massive exposure to the antigen. Secretory antibodies to *E. coli* develop differently depending on microbial exposure (21). Swedish infants had low levels of salivary SIgA antibodies against *E. coli* during the first 7 months of life whereas Pakistani infants showed a gradual increase and significantly higher levels. The levels of these antibodies in Swedish and Pakistani adults did not show any significant difference. Corresponding to the SIgA anti-*E. coli* levels in the Swedish infants, the SIgA anti-RSV levels in our study group could reflect low exposure.

Secretory antibodies against RSV showed a high avidity at birth, followed by a continuing decrease of avidity towards the end of the infants' first year of life. Breast milk antibody avidity was low, whereas maternal serum anti-RSV IgG showed a high avidity comparable to neonatal SIgA-anti RSV.

This finding could be readily explained by the concept that the neonate produces specific SIgA in response to anti-idiotypic IgG transferred from maternal serum to the fetus transplacentally and acting as a template for active SIgA synthesis (22). The affinity of this antibody would be expected to match serum-IgG anti-RSV rather than e.g. breast-milk antibody. Priming of the neonate by T-lymphocytes in breast milk seems improbable since SIgA antibodies were also found in neonates who did not receive breast milk.

In allergy-prone infants several types of abnormal immune responses can be seen. Elevation of cord blood IgE has been found to predict allergic disease with a high specificity (8, 9). Elevated SIgA anti-casein in saliva was also seen in infants who later developed atopic disease (4). In contrast, we found a deficient SIgA anti-RSV response in symptomatic high-risk infants at 1 year of age. This deficient response fits earlier findings of a deficient total SIgA in atopic children (23, 24).

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