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Ontogenesis of the formation of secretory antibodies to respiratory syncytial (RS) virus

N. P. LESCHINSKAYA, E. E. POKROVSKAYA,
E. A. KANTOROVITCH, S. K. GRIGORJEVA AND YA. S. SHVARTSMAN
All-Union Research Institute for Influenza, USSR Ministry of Health, Leningrad

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SUMMARY

Examination of sera from 184 children aged between 0 and 12 years and 161 adults revealed a close correlation between age and the level of humoral anti-RS virus immunity. Secretory IgG antibodies were found in children in their first months of life. Evidence for their release into secretions from the serum was obtained. This might explain the positive correlation between serum antibody levels in women recently confined with the morbidity due to RS virus in children during their first months of life. Secretory IgA antibodies were found from 4 months until old age. The secretions of children and adults contained virus-neutralizing activity which was non-immunoglobulin in nature, as well as antibodies. However, in contrast to secretory antibody this material did not prevent development of severe RS virus infections.

INTRODUCTION

Respiratory syncytial (RS) virus is the main cause of severe respiratory tract infections in newborn and children during the first year of life (Glezen *et al.* 1981; Hall, 1982; Hall *et al.* 1975; Mito, Hierholzer & Tannock, 1986). Recent clinico-epidemiological observations and immunological studies have shown the increasing role of RS virus in respiratory pathology in adults, especially in the elderly (Garvie & Gray, 1980; Sorvillo *et al.* 1984). A possible aggravating effect by maternal antibodies transmitted transplacentally on the clinical course of RS infection in children during their first months of life has to be re-evaluated. By inhibiting the formation of post-infectious immunity (Prince *et al.* 1983; Watt, Zardis & Lambden, 1986) these antibodies reduce the severity of primary RS infections and postpone the time of their appearance (Bruhn & Yeager, 1977; Ogilvie *et al.* 1981; Glezen *et al.* 1981; Ward *et al.* 1983). However, the mechanisms of protection provided by maternal antibodies remain undefined.

The second threat in the mechanisms of protection of newborns and children in the first year of life against RS infection is provided by new data on the decrease in prevalence and severity of the disease in breast-fed infants (Evans-Jones *et al.* 1978; Toms *et al.* 1980; Berman *et al.* 1983; Fishaut *et al.* 1981; Mito *et al.* 1984). This was confirmed by experiments in animals (Peri *et al.* 1982; Suffin *et al.* 1977; Prince *et al.* 1983).

In any understanding of the mechanisms of protection due to local antibodies in the respiratory tract, studies showing the presence of virus-neutralizing inhibitory substances which differ from specific IgA antibodies in the respiratory secretions of children at the age of 1–3 months must be included (Kim *et al.* 1969; Scott & Gardner, 1970, 1974; McIntosh *et al.* 1978). However, there have been hitherto no publications on the presence of inhibitors in the secretions of older children and adults and, what is more important, on the role of these inhibitors in resistance to the disease.

The aim of the present work was to study ontogenesis in the formation of secretory and serum antibodies to RS virus as well as the non-antibody virus-neutralizing inhibitors in the secretions of the upper respiratory tract in children and adults.

It was established that antibodies to RS virus present in the upper respiratory tract of children in the first 3 months of life were initially of IgG class and only later, IgA. Non-specific virus-neutralizing substances of a non-antibody nature were present in the secretions of both children and adults and were absent in sera. In contrast to antibodies, these substances were not involved in protection against infection.

MATERIALS AND METHODS

Groups under study

These were: 61 pairs of clinically healthy mothers and newborns examined during April and May 1985; 37 children between 0 and 3 months born and examined during June and August 1985 and without a history of acute respiratory disease (ARD); groups of 30 children aged between 4 and 36 months; 20 children between 5 and 12 years; 30 adults aged between 18 and 20 years and 30 adults over 50 years old; 36 children of nursery age hospitalized during the winter 1984–5 with RS virus infection. Upper respiratory tract infections (URI) were diagnosed predominantly in children over 1 year of age with nasopharyngitis of various degrees of severity, but mainly without fever. Lower respiratory tract infections (LRI) were diagnosed in children with acute lung failure and symptoms of bronchiolitis. Forty adult volunteers vaccinated with experimental live RS virus vaccine were also examined (Leschinskaya, Klimanova & Pokrovskaya, 1982).

Collection of secretions and sera

A soft catheter connected to an aspirator was inserted into the inferior nasal passages to collect mucus from the nasopharynx. Secretions were dispersed by an MSE ultrasonic homogenizer fitted with a small conical rod for 45–60 sec at 2·5–3 × 10 kHz and were separated from suspended particles by centrifugation for 30 min at 2000–3000 rev/min. The supernatant was stored at −25 °C with the addition of antibiotics until examined.

Serum from capillary blood collected from the finger was separated conventially and stored at −25 °C until examined.

Colostrum collected from women on the 2nd and 3rd days after delivery and milk collected from them later (on the 6th and 8th days) were separated from fat by centrifugation (VAC-602) at 40000 g for 1 h. Any residual fatty substances were removed by filtration through special paper filters for biological purposes.

Sera were stored at −25 °C until examined.

Antibody determinations

The antibody assays used were: a neutralization test in tissue culture (NT); indirect haemagglutination (IH) and indirect immunofluorescence (IF). The NT was a conventional one in tube monolayer cell cultures of HEp-2 cells with 50 TCD50 of the reference Long strain of RS virus. The titre of virus-neutralizing (VN) activity was defined as the highest dilution of the material under study which suppressed the cytopathic effect by 50% or more.

IH was a conventional one in microtitre plates using 50 ml volumes. The antigen used was prepared from the reference Long strain of virus and was conjugated on to formalinized erythrocytes by bi-diazotized benzidine (Shvartsman *et al.* 1977). Coombs' modified test was used to determine antibody isotypes (Coombs *et al.* 1965). IH was performed in three parallel rows and the erythrocytes were washed twice after reading the test. The settled cells were then resuspended, and previously selected dilutions of commercial antisera to the heavy chains of human IgG, IgM and IgA (produced by the Metchnikov Moscow Research Institute of Vaccines and Sera) were added to the wells. After thorough mixing of the ingredients the plates were kept at 37 °C for 2 h, then overnight at 4 °C after which the results were reassessed. After the addition of antiglobin a four-fold or greater increase in the titre of the material tested was considered a positive result and indicated that antibodies of the corresponding isotype were present. In the controls, the working dilutions of the antiglobulin sera were studied with the antigen and a suspension of non-sensitized erythrocytes. Similarly the minimal dilutions of the materials under study were assessed with a suspension of non-sensitized erythrocytes.

To determine antibodies by IF, polished glass rings with an inner diameter of 9 mm were attached to microscope slides. Growth medium (0·25 ml) containing 4×10^4 HEP-2 cells infected with RS virus were cultured in the chambers for 24–36 h until examined in the IF test. To allow the cells to attach to the surface of the slides the latter were placed in sterile Petri dishes which were incubated for 4–6 h at 37 °C. After incubation the medium was removed from inside the rings, the rings were removed, the cells washed once with the Eagle's medium and fixed with acetone. The 'spots' of cells obtained in this way were used to detect antibodies in sera and secretions by indirect IF. For this purpose cells were overlaid with twofold dilutions of the sera or secretions under test and were incubated for 30 min in a moist chamber at room temperature. Cells were twice washed with phosphate-buffered saline (PBS, pH 7·4), air-dried and stained for 30 min with fluorescent antisera to human globulins (produced commercially by the Gamaleya Research Institute of Epidemiology and Microbiology). Cell smears were twice washed in PBS for 10 min, rinsed with distilled water and air-dried with a hair drier at room temperature. Preparations were scanned under an ML-2 fluorescence microscope with filters SF 1-2, SF 1-4, SES 7-2.

Sera and secretions within a given age group were studied simultaneously.

Statistical methods

Statistical analysis in the groups of adults and children was done using Student's t test.

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Table 1. *Correlation between the results of RS infection antibody testing in volunteers by using IH, NT and indirect IF methods*

Materials under study	Compared tests	Correlation of results (%)	Confidence limits	Correlation factor	Geometrical mean titres of antibodies by IH	NT	IF
Sera	IH–IF	87·2	74·9–94·8	0·74			
	IH–NT	84·6	71·9–93·1	0·71	239	91	64
	NT–IF	87·6	74·9–94·8	0·74			
Secretions	IH–IF	88·0	73·1–95·8	0·75			
	IH–NT	53·0	36·1–68·4	0·24	22·6	13	8·6
	NT–IF	60·0	36·8–73·0	0·35			

Table 2. *Antibody by class in blood of women recently confined, umbilical cord blood of newborns, colostrum and milk (by IH)*

Material under study	Number studied	Number (%) with antibodies	Geometrical mean titres	Antibody isotypes in material under study (%) G	M	A
Maternal blood	61	44 (72·1%)	16·0	39 (63·9)	12 (19·7)	4 (6·6)
Umbilical blood	61	33 (54·1%)	13·9	33 (57·4)	0	0
Colostrum and immature milk	48	29 (61·7%)	3·7	3 (5·1)	0	29 (63·8)
Mature milk	51	24 (47·1%)	3·1	5 (9·6)	0	24 (53·8)

RESULTS

Comparison of NT, IHT and IF titres in sera and secretions

Thirty-nine serum samples and 40 samples of secretions collected from adult volunteers immunized with a live RS virus vaccine were assessed by three techniques. The serum antibody positivity rates by the three methods were similar (Table 1). However, in contrast to the data from serum antibodies, analysis of the results on secretions showed a low level of correlation between the data by IH and IF on the one hand, and by the NT on the other hand ($P < 0{\cdot}05$). At the same time there was a high correlation between the results of IH and the indirect IF method. IH was a slightly more sensitive method for investigating both serum and secretions than the NT and IF methods. The geometrical mean titres of serum antibodies by IH were 239, 91 by NT and 64 ($P < 0{\cdot}05$) by IF; while the equivalent geometrical mean titres of secretory antibodies were 22·6; 13 and 8·6, respectively.

Inhibitory and virus-neutralizing activity of a non-immunoglobulin nature in blood sera and secretions of children and adults

Antibodies to RS virus were revealed in the sera of the majority of recently confined women. They were transmitted transplacentally to more than half the

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Table 3. *Concentration of neutralizing activity (by NT) and antibodies (by IH) to RS virus in blood and respiratory secretions in children and adults*

		Blood (1:8)				Secretions (1:2)			
		NT		IH		NT		IH	
Age	Number of subjects under study	Number (%) of positive samples	GMT* (M±S.D.)	Number (%) of positive samples	GMT* (M±S.D.)	Number (%) of positive samples	GMT* (M±S.D.)	Number (%) of positive samples	GMT* (M±S.D.)
0–3 months	37	11 (30·0)	4·6±0·38	16 (42·1)	7·0±0·28	16 (42·1)	2·6±0·70	12 (31·6)	1·8±0·04
4–36	30	15 (50·0)	4·3±0·34	23 (76·6)	4·7±0·05	16 (53·3)	2·6±0·70	9 (30·0)	1·7±0·04
5–12 years	20	16 (80·0)	8·6±0·18	20 (100·0)	11·3±0·21	14 (70·0)	2·6±0·12	12 (60·0	3·5±0·14
18–20	30	30 (100·0)	28·0±0·25	30 (100·0)	30·0±0·25	14 (46·7)	3·1±0·20	22 (73·3)	4·3±0·19
50 years and over	30	30 (100·0)	28·0±0·25	30 (100·0)	32·0±0·39	21 (70·0)	3·3±0·14	26 (86·7)	6·5±0·27
Total	148	102 (68·9)		103 (69·6)		81 (54·7)		81 (54·7)	

* GMT, geometrical mean titres±standard deviation.

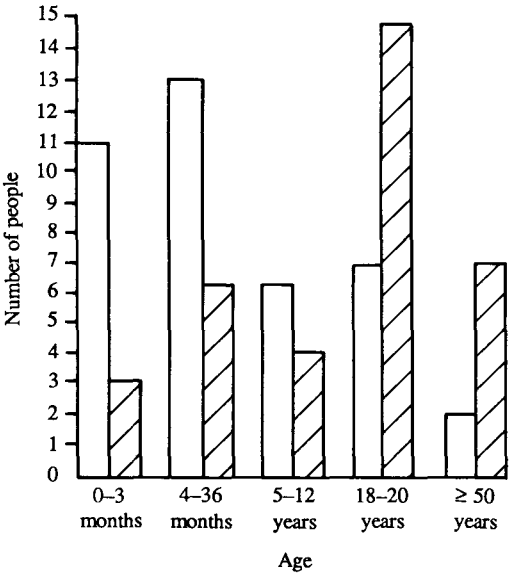

Fig. 1. Neutralizing activity and antibodies to RS virus in blood of children and adults. □, Neutralizing activity (only by NT); ▨, antibodies (only by IH).

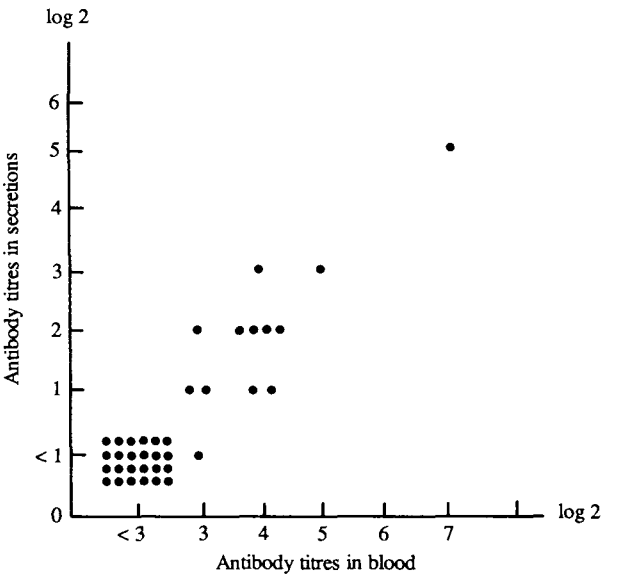

Fig. 2. Correlation of antibody concentration in blood sera and secretions of newborns.

newborns. Antibodies to RS virus were found also in colostrum and breast milk (Table 2).

There was a correlation between the age of the patient and the frequency of RS virus antibodies in the sera (Table 3). In subjects 18 years old and over, antibodies were found in 100% of cases by NT and IH. The geometrical mean titres had

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Table 4. *Antibody by class in sera and secretions of clinically healthy children and adults*

	Sera						Secretions					
	Number of subjects with	Class					Number of subjects with	Class				
Age	antibodies	G	M	A	G+M	G+A	antibodies	G	M	A	G+A	M+A
0–3 months	15	16 (100%)	0	0	0	0	12	12 (100%)	0	0	0	0
4–36	23	17 (73·9%)	2 (8·7%)	0	4 (17·4%)	0	9	0	0	7 (77·8%)	2 (22·2%)	0
5–12 years	20	9 (45%)	3 (15%)	0	3 (15%)	5	12	0	0	10 (83·3%)	2 (16·7%)	0
18–20	30	26 (86·7%)	0	0	0	4 (13·3%)	28	0	0	22 (78·6%)	15 (17·9%)	0
50 years and over	30	25 (83·3%)	5 (16·7%)	0	0	0	26	0	0	26 (100%)	0	0

Table 5. *Effect of secretory IgA antibodies and neutralizing activity of non-immunoglobulin nature on the course of RS infection*

Localization of lesions	Number of children	Number (percentage) of subjects with antibodies on admission to hospital: Antibodies	Antibodies + virus-neutralizing activity*	VNA
URTI†	14	4 (28·1)	7 (50·0)	0
LRTI‡	22	0	0	9 (40·9)

* Virus-neutralizing activity of a non-immunoglobulin nature.
† Upper respiratory tract infection.
‡ Lower respiratory tract infection.

reached maximum values by this age. More frequent positives detected by IH than by NT in children were due to the greater sensitivity of IH.

Similar data on the acquisition of anti-RS virus immunity with age were obtained from the investigation of respiratory secretions by IH but not by NT. Analysis of the results showed that each age group under study included subjects who had virus-neutralizing activity but who had no antibody detectable in secretions by IH (Fig. 1).

Of special interest were the data on serum and secretory antibodies in 37 children aged up to 3 months who were born in the summer (the inter-epidemic period for RS virus). Antibodies in their sera were IgG, and antibodies in the secretions from the upper respiratory tract belonged to the same isotype.

It is important to note that antibody titres in the sera of newborns who were born in the inter-epidemic period correlated with antibody titres in their secretions ($P = 0{\cdot}949$) (Fig. 2).

In children over 4 months old specific IgG and IgM antibodies began to appear, but secretory antibodies were of the IgA class. IgM-class antibodies were found in the sera of some of those in the older age groups (Table 4).

Secretory antibodies and substances of a non-antibody nature in resistance to RS infection

Among the children aged between 5 and 11 months who were hospitalized because of serologically proved RS infection were some patients who already had specific secretory antibodies on admission to hospital or who had both antibodies and non-specific virus-neutralizing activity (by NT). The course of the disease in them (Table 5) was mostly mild and mainly confined to the upper respiratory tract. In contrast, the presence or absence of the non-immuno-globulin neutralizing material on admission to hospital did not influence the severity of the disease.

DISCUSSION

Specific prevention of RS infection in children and in a definite part of the adult population is the major component in the medical control of viral ARD in man at

present (Anon., 1984; Hall, 1980; Pringle, 1987). As a basis for solving this problem, it is necessary to study ontogenic mechanisms of anti-RS virus humoral immunity and also the problem of non-immunoglobulin virus-neutralizing activity in the secretions of the upper respiratory tract.

The work reported here showed the principal difference in the characteristics of secretory antibodies in children in the first months of life, who were born in the inter-epidemic period of RS virus infection, and children over 4 months old.

The discovery of secretory IgG antibodies in children of the first group, the presence of the similar antibody class in the sera of women recently confined and in cord blood, and the presence of a good correlation between the level of serum and secretory antibodies in children during the first 3 months of life, is consistent with the assumption that the mucosa of the upper respiratory tract have an increased permeability at that period. For the same reason transplacental antibodies penetrate into secretions as well. This hypothesis provides a satisfactory explanation of the positive correlation between the level of antibodies in maternal sera and the resistance of newborns to RS virus infection. The applied consequence of this would be the advisability of immunizing pregnant women with an RS virus vaccine to increase the resistance of newborns to this infection.

The other source of anti-RS virus antibodies in children during the first months of life is breast milk. Anti-RS virus antibodies in this secreted material belonged to the IgA class.

Secretory IgA antibodies are found from 4 months of age until old age, and this confirms that they are synthesized as a result of infection. Repeated infections mean that specific antibodies are already present in the sera and secretions of the upper respiratory tract in the majority of children examined at school age.

The presence of IgM-class antibodies in the sera of elderly people proves that re-infection with RS infection can occur at this age, and makes it possible to immunize elderly subjects with serious cardiovascular and bronchopulmonary lesions against RS virus infection. The results of these studies confirmed the data of McIntosh *et al.* (1978) showing the presence of virus-neutralizing activity of a non-antibody nature in the secretions of the upper respiratory tracts of children in the first months of life. In addition they showed that such neutralizing activity is also found in the upper respiratory tract secretions in older children and in adults.

To develop a practical way to prevent RS infection it is important to understand the role of non-specific virus-neutralizing activity in the upper respiratory tract secretions in resistance to the disease. The examination of children hospitalized because of RS infection showed that non-antibody virus-neutralizing substances are not involved in the control of the infectious process at the stage of its onset. In contrast, secretory IgA antibodies play a leading role in this process. As for the role of secretory antibody for the severity of RS infections in young children, the data obtained in our investigation correlate fully with the results published by Scott & Gardner (1974).

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