

ANTIGENIC AND BIOLOGICAL CRITERIA OF DIFFERENTIATION OF THE NEWLY ISOLATED STRAINS OF RESPIRATORY SYNCYTIAL VIRUS

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Received June 4, 1980; revised June 6, 1982

Summary. — Respiratory syncytial (RS) virus strains isolated in different years varied by their antigenic and biological properties. The lowest degree of relatedness was found between the “street” virus and the prototype Long strain; the highest occurred among the isolates from a given isolation period. Based on the mean indices of efficiency of the virus reproduction in human embryo lung (HEL) cells at 37 °C and 39 °C as well as on the degree of virus sensitivity to reference antibodies, the isolates from various years could be divided into three groups, namely high, mild and low virulent strains. The incidence of RS virus infections in children depended on the strain characteristic of virus population circulating in a community of children during the long-term observation period of 1976—1979. Cyclic variation was found in isolation rates of RS viruses; the duration of each cycle in different years ranged from 21 to 41 days. The variability of isolation cycles and the frequency of RS virus reinfections were closely related to the biological characteristics of circulating virus strains.

Key words: respiratory syncytial virus; antigenic variation; virus variability; isolation cycle

Introduction

Antigenic diversity of RS virus strains isolated in different years has been demonstrated by several authors (Coates *et al.*, 1963, 1966; Wulff *et al.*, 1964; Monto *et al.*, 1974; Hierholzer, 1979). Despite of a wide distribution of RS viruses, no detailed data are available on the degree of variation of their antigenic and biological properties in the course of epidemics.

The purpose of the present paper was to find out criteria determining the degree of virulence of RS viruses isolated in different periods of epidemic seasons.

Materials and Methods

Diagnosis of RS virus infection. A community of 100 children from the birth to 3 years was followed up regularly for cases of acute respiratory disease (ARD) during the period of three years (October, 1976 — December, 1979). Attempts at RS virus isolation were performed in

different epidemic seasons (autumn, winter and summer). The methods of virus isolation and identification were described in our previous paper (Yurlova *et al.*, 1983). Serological diagnosis of RS virus infection was carried out by the complement-fixation (CF) test among children with and without ARD (monthly serological observation).

Antigenic characterization of isolated RS virus strains. The virus was grown in HEL cells cultured in Eagle's medium supplemented with glutamine. The virus was separated from the cell detritus by centrifugation at 3,000 rev/min. Guinea pig immune sera containing antibodies to RS virus were prepared as described (Yurlova *et al.*, 1983). The sera were inactivated by heating at 56 °C for 30 min and treated by rivanol. For antigenic characterization of the isolated strains, virus neutralization (VN) test was employed using two-fold dilutions of immune sera and 100 infectious doses (ID₅₀) of the homologous or heterologous virus strains. The mixtures of equal volumes of sera and viruses were preincubated for 2 hr at room temperature prior to the inoculation into HEL cells. The cells were incubated at 36 °C for 5–7 days, until a complete cell destruction was observed. The serum dilution which completely prevented the cell destruction was considered for the VN titre.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
1	256											1.2
2		256										1.8
3			128									1.1
4				128								0.9
5					128							0.8
6						128						1.5
7							128					0.81
8								128				1.5
9									64			2.6
10										256		1.6
11											128	1.8
12	9.4	13.2	50.0	53.8	45.6	26.5	18.7	19.6	68.7	56.2	50.0	

Fig. 1.

Antigenic characterization of different RS virus strains isolated in various periods of epidemic seasons

Virus strains and their isolation dates:

1 — prototype Long; 2 — RS Leningrad 079/76 (4.11. 1976); 3 — RS Leningrad 247/76 (16. 12. 1976); 4 — RS Leningrad 391/77 (20. 1. 1977); 5 — RS Leningrad 577/77 (10. 3. 1977); 6 — RS Leningrad 889/77 (2. 6. 1977); 7 — RS Leningrad 1053/77 (21. 7. 1977); 8 — RS Leningrad 1102/77 (18. 8. 1977); 9 — RS Leningrad 1167/77 (27. 10. 1977); 10 — RS Leningrad 1192/77 (15. 11. 1977); 11 — RS Leningrad 1264/77 (15. 12. 1977); 12 — degree of relatedness (in %).

Immune sera prepared to each virus strain; I = antiserum to the prototype Long strain; II — XI = antisera raised to the strains listed under Num. 2 — 11; XII — CRNI (the coefficient of regression of neutralization index).

Homologous serum titres (serum dilution reciprocals) are given for each virus strain reacting with the corresponding antibody. Heterologous serum titres were expressed as proportions of the homologous titre (in %, black areas of each square).

Notice: the isolates differed less according to their sensitivity to antibodies than according to their antigenic characteristics, which confirms their true antigenic variations.

Table 1. Differentiation of 150 freshly isolated strains of RS virus according to the mean values of their reproduction and the degree of their sensitivity of specific antibodies

Virulence of virus strains	Mean values ($M \pm m$) of the coefficients of regression			Per cent deviation from the mean values of		
	of infectious activity (CRIA) ¹		of neutralization indices (CRNI) ²	CRIA		CRNI
	at 37 °C	at 39 °C		at 37 °C	at 39 °C	
High	2.3 \pm 0.23*	2.8 \pm 0.22	0.99 \pm 0.27	10.9	7.8	27.2
	199.5 \pm 17.0**	660.7 \pm 16.0	9.7 \pm 1.8			
Mild	1.5 \pm 0.2	1.95 \pm 0.24	1.67 \pm 0.15	10.2	10.9	8.9
	31.62 \pm 16.0	89.13 \pm 17.0	46.77 \pm 14.0			
Low	0.98 \pm 0.1	1.08 \pm 0.25	2.56 \pm 0.23	9.8	23.1	8.6
	9.5 \pm 5.0	12.02 \pm 3.0	363.1 \pm 3.0			

* The rate of virus reproduction in Ig units per day. ** The same in anti-Ig units per day
¹, ² = for explanation see this volume pp. 250–255.

Results

As follows from our investigations, the antigenic characteristics of RS virus strains isolated in different years were far from being related to each other. The majority of our isolates reacted with the reference serum prepared against the prototype Long strain in 4- to a 5-fold lower dilutions as compared to the homologous serum titre, indicating a marked antigenic difference between strains circulating in the period of our investigation and the prototype virus strain. The degree of relatedness of the "street" virus strains with the prototype strain was expressed as a proportion (in %) of the mean geometric VN titre of sera tested with newly isolated virus strains to that obtained with the homologous virus strain. The lowest degree of relatedness of "street" virus strains (9.4%) was found against the prototype Long strain, the highest one among the isolates from a given investigation period. However, newly isolated strains contained antigenic determinants of the prototype Long strain, because it was neutralized with immune sera prepared to the new isolates in the same titre as with the homologous anti-Long serum.

The characteristic peculiarity of RS virus infections was the possibility of parallel circulation under natural conditions of older as well as more recent antigenic virus variants. The degree of relatedness of RS virus strain Leningrad/079/76 with the prototype Long strain was 100%, whereas with later isolates (from December, 1976 to December, 1977) it was only 13.2% (Fig. 1). Antigenic analysis of newly isolated strains revealed that population of RS virus strains from one epidemic season was sufficiently homogeneous. It differed somewhat from the strains of another epidemic season and markedly from the virus variants isolated in summer.

Data on differentiation of RS virus isolates according to some genetic markers, namely the activity of virus reproduction in cell cultures and the degree of virus susceptibility to specific antibodies are presented in Table 1. To each virus group corresponded a certain mean value of the coefficient

Table 2. Variability of the duration of virus isolation cycles and of the frequency of RS virus reinfections in different epidemic season periods

Period of investigation	Incidence of		Period of virus isolation		Incidence of reinfections		Incidence of severe forms of ARD*	Incidence of asymptomatic forms of ARD*
	virus isolation	serological diagnosis	1-7 days	21-41 days	virus isolation	serological diagnosis		
Oct. 76— Sept. 77	47/117* 40.1%	87/113 76.9%	32 68.0%	15 31.9%	20/117 17.0%	22/113 19.4%	18/81** 22.2%	5/81** 6.1%
Oct. 77— Sept. 78	27/103 26.2%	47/98 47.9%	24 38.0%	3 11.1%	3/103 2.9%	5/98 5.1%	6/46 13.0%	9/46 19.5%
Oct. 78— Oct. 79	19/91 20.8%	61/127 48.0%	12 63.1%	7 36.8%	3/91 3.2%	15/127 11.8%	6/61 9.8%	10/61 16.3%

* The number of children under observation.

** The number of children with confirmed ARD laboratory.

of regression of infectious activity (CRIA) in cells incubated at 37 °C or 39 °C as well as the coefficient of regression of neutralization indices (CRNI), i.e. the dose of neutralized virus (in lg units) per dilution unit of the homologous immune rabbit serum (Yurlova *et al.*, 1983). Dispersion (S) of the latter coefficient was fully admissible, because deviation from mean values varied from 8.6 to 27.2%. The activity of reproduction of high virulent virus strains almost three times surpassed that of low virulent isolates. High virulent virus strains were at the same time less sensitive to specific antibodies.

Seroepidemiological and virological analysis of the outbreaks of ARD connected with RS virus infections in a children community during the three years' investigation period revealed that the outbreaks had differed as to their dynamics, intensity, duration, and possibility of reinfection of the same individuals. As shown in Table 2, incidence of ARD depended on the strain characteristics of viruses circulating in different epidemic seasons varying from 48.0 to 76.9% and from 20.8 to 40.1%, based on serological and virological diagnosis, respectively. Per cent morbidity during an outbreak caused by more virulent RS virus strains isolated in 1976–1977 was 18.8, ranging from 10 to 41, i.e. it was 2.5 times higher than that during outbreaks occurring in 1977–1979.

Clinical picture of ARD observed in different years also differed: severe or complicated forms of RS virus infections were more frequent during the 1976–1977 outbreak, whereas asymptomatic forms were more frequent in 1977–1979. The frequency of repeated cases of ARD in association with RS virus reinfections depended on the extensity of virus distribution in different epidemic seasons, being the highest in the period from October, 1976 to September, 1977, and the lowest in the period from October, 1977 to September, 1978, respectively (Table 2).

As follows from an analysis of the duration of successful virus isolation from the nasopharynx of sick children, the RS virus infection is characterized by a certain periodicity of positive isolation. The duration of one cycle ranged from 21 to 41 days, the phase of active isolation (2–7 days) within this range being followed by the phase of relative refractoriness (2–4 weeks). During the latter, activation of endogenous virus such as latent herpes- and adeno-infections, was often observed. Both apparent and asymptomatic forms of RS virus infection occurred in the followed-up children group. In general, acute forms were usual in the beginning of the cycle, less often in the beginning and at the end of the observation period. Table 2 also presents data on the duration variability of the RS virus isolation cycles corresponding to the characteristics of virus strain which circulated during different periods of the epidemic season. Thus, the maximum duration of RS virus isolation as found from the beginning of ARD was 41 days in 1976–1977, but only 21 days in 1978–1979.

Discussion

Numerous investigations using other viruses showed a sufficient diversity among the circulating virus populations. Even the same virus strain en-

counters complicated intrapopulation relationships between infectious and non-infectious virus particles leading to the selection of viruses yielding high, mild and low harvests. It is assumed that infectious virus particles may play the role of "helpers" in the synthesis of non-infectious particles, which in turn may inhibit the replication of infectious virus. Thus, not only genetic but also dynamic variations in the composition of circulating virus populations may occur, caused by preferential selection and choice of variants with a wide spectrum of virulence (Kantorovich-Prokudina, 1976).

As follows from our investigation, populations of RS virus strains isolated in different years differed in their biological and antigenic properties. It is supposed that genetic markers determine only indirectly the degree of virus virulence, because the correlation between certain genetic markers and virus pathogenicity is far from to be absolute and often is connected only with a special strain characteristic.

Genetic markers used for determination of the strain characteristic of RS viruses in our study made it possible to classify the new isolates of RS virus into three groups, namely high, mild and low virulent virus strains. The results obtained confirmed a close interrelationship between the biological characteristic of circulating virus strains and their epidemic activity. According to our opinion, the maximum frequency of severe and complicated forms of ARD, the high frequency of RS virus reinfections, the maximum infectivity, the prolonged duration of virus isolation and circulation in the community under study as well as the high morbidity, all these characteristics of RS virus infections observed from October, 1976 to September, 1977, were caused by circulation of virus strain(s) of highest virulence.

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