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SOME REGULARITIES IN VARIATIONS OF RESPIRATORY SYNCYTIAL VIRUS STRAINS DURING THEIR EPIDEMIOLOGICAL CIRCULATION

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Summary. — Among respiratory syncytial (RS) viruses circulating in the territory of U.S.S.R., strain variations were observed based on the differences in activity of virus reproduction in human embryo kidney cell cultures at 39 °C and in virus sensitivity to antibodies. Mathematical expression of the correlation between these two genetic markers made it possible to classify the new isolates of RS virus into three groups differing in their virulence, namely high, mild and low virulent strains. Populations of RS virus strains varied depending on the epidemic season: during the first period (1976—1977) predominated the high virulent (52—78%) while during the second period (1977—1978) the mild virulent (55—65%) strains. The third period (1978—1979) was characterized by the circulation of a mixture of mild and low virulent strains. In summer low virulent virus strains prevailed (56—89%).

Key words: *respiratory syncytial virus*; *virus population*; *genetic markers*

Introduction

Many data indicate that variation of viruses under natural conditions includes diversity of their biological properties, namely of their virulence. Virulence variation among virus strains is known for a wide spectrum of viruses circulating in nature, such as togaviruses, orthopoxviruses, parapoxviruses, cytomegaloviruses, and paramyxoviruses (Albrecht and Weller, 1976; Borsuk *et al.*, 1978; Hayes, 1978; Tantawi *et al.*, 1978; Trent *et al.*, 1978; Wittek *et al.*, 1978). To determine the virus virulence either in the process of cold-adaptation to cell cultures of different origin or during natural variation, several genetic markers have been elaborated, such as rct_{40} — activity of virus reproduction at 40 °C, s — size of virus plaques, t — thermoresistance, if — interferon inducing ability, etc. However, no absolute correlation between individual genetic markers and pathogenicity of viruses has been found, the latter often being only an expression of the special property of given virus strain.

The purpose of this paper was to present data on criteria of differentiation among populations of newly isolated RS virus variants.

Materials and Methods

Virus isolation. For virus isolation, nasal and nasopharyngeal swabs were collected from children with acute respiratory diseases (ARD) and from contact children without ARD (almost weekly virological observation). Cell cultures (primary human embryo kidney cells and human diploid lung cells) were inoculated with 0.2 ml volumes of the virus containing material. The presence of virus in cells was determined in cell cultures according to the cytopathic effect. Identification of the newly isolated virus strains was performed by neutralization test in cell cultures using corresponding monospecific antisera.

Determination of infectious activity of the virus. Serial ten-fold dilutions of the virus were inoculated into tubes containing monolayers of human embryo kidney cells and incubated in parallel at 37 °C and 39 °C, respectively. The virus titres were calculated according to Reed and Muench.

Determination of the efficiency of virus reproduction. The rate of virus reproduction was determined daily using the method of regression analysis, i.e. by least square fitting expressed in coefficients of regression of infectious activity $CRIA_{39}$ or $CRIA_{37}$ (Rokicky, 1967; Selivanov *et al.*, 1972). The coefficient of regression estimated for each virus strain at given temperature reflected the slope of virus reproduction sequence. The higher was the value of regression coefficient, the higher was the ability for reproduction of the virus strain tested.

Determination of the degree of susceptibility of viruses to specific antibodies. For each virus strain under study the regression (neutralization) line reflecting the character of interaction between different dilutions of immune rabbit serum (in $\log_2$ units) and ten-fold dilutions of the virus (the square root mode) was determined. The reference Long strain was used for immunization of rabbits. To each dose of immune serum corresponded a given value of neutralization index. The mean value of neutralization index (the number of virus doses neutralized per unit of serum dilution), which was shown to be a stable indication of virus neutralization, was expressed by coefficients of regression of neutralization indices — CRNI (Kovalieva, 1974). To determine neutralization indices, the method of inhibition of the cytopathic effect of RS virus isolates by specific rabbit immune sera prepared to the reference Long strain was employed.

Results

Seasonal variations of RS virus strains

The RS virus strains isolated during the three epidemic seasons differed in the dynamics and rate of their reproduction in human embryo kidney cells at 39 °C.

The most of RS virus population circulating during epidemic season 1976—1977 was characterized by high indices of the reproduction rate ($2.5 < CRIA_{39}$), while virus strains from the period of 1977—1978 were of lower infectivity ($1.5 < CRIA_{39} \leq 2.5$); the population circulating in 1978—1979 consisted of a mixture of low and mild growing virus strains (Table 1). In summer, the low yielding virus strains predominated ($CRIA_{39} \leq 1.5$). Similar results were obtained when analysing the efficiency of reproduction of different virus strains among virus populations by determining the infectious doses harvested per incubation day, e.g. low yielding strains did not produce on the average more than 50 infectious doses, but the high yielding ones produced more than 300 infectious doses per cell and incubation day.

The RS virus strains isolated in different periods of epidemic seasons differed also in the degree of their sensitivity to specific antibodies. Based on the absolute values of CRNI, the newly isolated virus strains could be again classified into three groups: strains with high, medium and low re-

Table 1. Differentiation of newly isolated strains of RS virus according to the activity of their reproduction in human embryo kidney cell cultures at 39 °C

Period of investigation	Number of strains	Absolute numbers of virus strains with given reproduction activity $CRIA_{39}$ intervals						
		< 1.0	1.0—1.5	1.6—2.0	2.1—2.5	2.6—3.0	3.1—4.0	> 4.0
November, 1976	1			1				
December, 1976 — — April, 1977	46	2	5	5	10	17	5	2
June—August, 1977	9	6	2		1			
September — November, 1977	6		1		3	1	1	
December, 1977 — April, 1978	20	2	1	6	7	4		
October, 1978 — March, 1979	38	8	16	11	3			
Total	120	18	25	23	24	22	6	2
Strain yield*		low $CRIA_{39} \leq 1.5$		medium $1.5 < CRIA_{39} < 2.5$		high $2.5 < CRIA_{39}$		

* Expressed as the coefficients of regression of infectious activity at 39 °C ($CRIA_{39}$) in log units/day.

activity with the reference strain antibodies. Populations of epidemic virus strains were of low reactivity in the period of 1976—1977 and of medium reactivity in the period of 1977—1978, respectively. In summer quite often occurred strains with a high reactivity (Table 2).

Our studies revealed an inverse proportion between the intensity of virus reproduction in cells cultivated at 39 °C and the degree of their reactivity

Table 2. Differentiation of newly isolated strains of RS virus according to their sensitivity to specific antibodies

Period of investigation	Number of strains	Absolute numbers of virus strains with given sensitivity to reference antibodies (CRNI intervals)*							
		< 1	1.1—1.4	1.5—1.8	1.9—2.0	2.1—2.3	2.4—2.6	2.7—3.0	> 3.0
November, 1976	1			1					
December, 1976 — — April, 1977	46	17	19	7	1		2		
June — August, 1977	9	1		4	1		1	2	
September — November, 1977	6	2	1	2			1		
December, 1977 — April, 1978	20	5	3	6	5	1			
October, 1978 — March, 1979	38	3	5	9	7	6	4	2	2
Total	120	28	28	29	14	7	8	4	2
Antibody reactivity of virus strains*		low CRNI ≤ 1.4		medium 1.4 < CRNI ≤ 2.0			high 2.0 < CRNI		

* Expressed as the coefficients of regression of neutralization indices (CRNI).

Table 3. Characterization according to genetic markers of RS virus strains circulating in nature and isolated in different periods of epidemic seasons

Period of investigation	Seasonal characteristics	Number of virus strains	Characterization of virus strains: high virulent $2.5 < CRIA_{39}$ $CRNI \leqq 1.4$		medium virulent $1.5 < CRIA_{39} \leqq 2.5$ $1.4 < CRNI \leqq 2.0$		low virulent $CRIA_{39} \leqq 1.5$ $2.0 < CRNI$	
November, 1976	preepidemic	1			1	1		
December, 1976—April, 1977	epidemic	46	24 **52%**	36 **78%**	15 33%	8 17%	7 15%	2 4%
June—August, 1977	interepidemic	9		1	1	5 **56%**	8 **89%**	3
September—November, 1977	preepidemic	6	2	3	3	2	1	1
December, 1977—April, 1978	epidemic	20	4	8	13 **65%**	11 **55%**	3	1
October, 1978—March, 1979	epidemic	38		8	14 **37%**	16 **42%**	24 **63%**	24 **37%**
Total		120	30 25%	56 47%	47 39%	43 36%	43 36%	21 17%

with reference strain antibodies. The correlation between these two markers was sufficiently high (the correlation coefficient was 0.82). Mathematical expression of the correlation made it possible to differentiate the newly isolated RS virus strains into three groups according to their virulence: high, mild and low virulent. As shown in Table 3, the high virulent strains possessed a high coefficient of regression ($2.5 < CRIA_{39}$) and a low reactivity with antibodies ($CRNI \leqq 1.4$); in contrast, the low virulent strains had a low coefficient of regression ($CRIA_{39} \leqq 1.5$) but a high reactivity with antibodies ($2.0 < CRNI$). Finally, the mild virulent strains could be placed in between, showing an intermediate coefficient of regression ($1.5 < CRIA_{39} \leqq 2.5$) as well as medium reactivity with the reference antiserum ($1.4 < < CRNI \leqq 2.0$).

Two genetic markers of RS virus strains circulating in nature

The results obtained indicate a possible dynamic relationship among natural RS virus strains. This was demonstrated by qualitative and quantitative changes in composition of virus populations ranging from high to low virulent strains depending on the period of investigation. As follows from Table 3, the RS viruses isolated in the period of 1976—1977 belonged according to the two genetic markers in question to the group of high virulent strains in 52—78% of cases, while those isolated in the period of 1977—1978 fitted in the group of mild virulent strains in 55—65% of cases; strains from the period of 1978—1979 belonging to the intermediate group consisted of a mixture of mild and low virulent virus populations.

Discussion

Repeated experimental investigation revealed the possibility of obtaining the virus variants possessing a wide spectrum of virulence. The whole number of genetic markers was elaborated enabling to differentiate the attenuated from original virulent strains of different viruses.

No strain variations as to the virulence of circulating strains of RS virus have been known as yet. The only data so far known concerned the possibility of obtaining areactogenic strains of RS virus based on the use of ts-mutants (Chanock *et al.*, 1975; Faulkner *et al.*, 1976; Belshe *et al.*, 1977; 1978) or the cold-adapted variants (Friedewald *et al.*, 1968; Leshinskaya *et al.*, 1972). These low virulent strains differed from original viruses in several markers tested, such as the activity of reproduction under conditions of permissive and non-permissive cultivation temperatures, the intensity of formation of filamentous protrusions on the surface of infected cells, the time and duration of their isolation from the human respiratory tract. However, at present no data are available on the qualitative composition of circulating RS virus populations in individual population samples and on the periodical change of virulent variants of RS virus collected at different periods of investigation. In our studies we postulated the hypothesis that under natural conditions of circulation, the causative agents of ARD could be high as well as low virulent virus strains and that the epidemic process caused by these

virus popoulations should be of relatively serious or easy course. We suppose that the genetic drift is based on the phenotypic changes of virus populations which reflect to one or another degree the character of changes of their genotypic composition.

Results of our investigations showed that some biological properties of RS virus changed during the epidemic process, such changes involving the number of genetic markers of the virus. We assume that data from comparative analysis of quantitative characteristics of the rate of infectious process in the cells cultivated at 39 °C and of the virus susceptibility to specific antibodies can be used as hopeful prognostic criteria evaluating the intensity of development of epidemic process in RS virus infections.

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