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DYNAMICS OF SOME GENETIC MARKER CHANGES OF RESPIRATORY-SYNCYTIAL VIRUS STRAINS CIRCULATING AMONG NURSERY-SCHOOL CHILDREN

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Summary. — Respiratory-syncytial (RS) virus strains circulating during several years appeared polymorphic in respect of two genetic markers: the regression coefficient of infectious activity ($RCIA_{39}$) characterizing the isolates by their reproduction in tissue cultures at supraoptimal temperature (39 °C) and the regression coefficient of neutralization indices (RCNI) characterizing the degree of sensitivity of the strains to antibodies. High-yield RS viruses were more often isolated from children frequently afflicted by the disease, moderate-yield viruses from moderately sick children, while low-yield or none-yield (at 39 °C) strains were isolated from rarely afflicted children. On the other hand, RS strains of low reactivity with prototype antibodies were mainly found in often or moderately sick children and the high-reactive ones in rarely sick children. The variability of the RS virus population was continuous, which is consistent with the uninterrupted course of the epidemic process in the nursery-school community. A change of the $RCIA_{39}$ marker was observed in nearly 50% of strains already after $1^1/_2$ to 2 months, but most frequently within 5—6 months from the end of disease. The changes of growth intensity at 39 °C followed the pattern: high → → moderate → low → none, however, in the next epidemic season these properties showed reversion in an opposite direction.

Key words: respiratory-syncytial virus; population; genetic markers; natural variability

Introduction

It is known that evolution depends on the changes of genetic composition of populations. Several factors contribute to principal evolution forces responsible for changes of the populations balance: mutation-recombination processes, the gene flow and drift as well as the selection factors. The level of biological adaptation of the pathogen is determined by the selection factor. According to Tsilinsky (1982), the RS virus population has a low evolution plasticity owing to its low recombination capacity. However, the

RS virus population is no exception from the viral kingdom; it undergoes mutations which provide an enormous variability. The genetic heterogeneity of populations is related to their interstrain and intrastrain variations. It should be emphasized that the time course of the populations is determined not only by genetic changes of their composition, but also by the intrapopulation phenotypic interactions. The intrapopulation relationships of infectious and non-infectious defective interfering (DI) particles within one strain are of especial importance, since the infective particles act as helpers in the synthesis of DI-particles. During their reproduction, these particles exert a blocking effect on the reproduction of the infectious virus (Kantorovich-Prokudina *et al.*, 1976). The complicated interactions within the population may result in production of clones with different biological properties; fixation of these properties in the subsequent generations is related to both the self-regulating properties of virus populations and the selecting influence of the macroorganism.

The purpose of this work has been to study the specific interactions of the heterogeneous host with the heterogeneous pathogen based on evaluation of interstrain genetic marker differences of the RS virus.

Materials and Methods

The observations have been conducted over many years in the group of young-age nursery-school children (less than 3-year-old) in which an aetiological diagnosis was regularly made in each case of acute respiratory disease.

RS virus was isolated and typed in diploid human embryo lung (HEL) cells or in the primary cell culture of human embryo kidney (HEK) using conventional techniques.

Estimation of virus reproduction efficiency. Reproduction rate of the viruses was measured in HEK cells at 39 °C, calculated by regression analysis using least-square techniques and expressed as regression coefficients of infection activity $RCIA_{39}$ (Rokitsky, 1967; Selivanov *et al.*, 1972). The biological sense of the $RCIA_{39}$ marker is the average quantity of the virus yield (log) per each or averaged day of incubation.

Estimation of the sensitivity of viruses to antibodies. The reactions of the virus strains with antibodies were determined on the basis of the neutralization of the isolates in tissue culture with immune rabbit sera to the reference Long strain followed by the regression analysis of the neutralizing activity of the sera expressed as neutralization indices. The marker RCNI is an averaged value of the neutralization indices, i.e. the number of neutralized virus doses per unit of 2-fold dilution of the serum (Kovaleva, 1974, Yurlova *et al.*, 1983). The blocking activity of the immune sera was determined by the intensity of 100% inhibition of cytopathic effect of the isolates in cell cultures.

Based on epidemiological studies the group of continuously observed children have been classified into a few subgroups: often, moderately and rarely afflicted by the disease, considering the annual acute respiratory disease morbidity in each age group.

Results

Kinetics of natural viral populations with respect to the $RCIA_{39}$ marker

Natural populations of RS viruses isolated in different years (1976—1981) differed in the intensity of reproduction in HEK cells at supraoptimal temperature (39 °C). High-yield viruses showed $RCIA_{39} \geq 2.5$, the moderate yield ones $1.5 < RCIA_{39} < 2.5$, the low-yield strains $1.0 \leq RCIA_{39} \leq 1.5$

Table 1. Specific features of the interaction of macropopulation (host) and micropopulation (pathogen) as related to the character of natural variability of RS viruses with respect to the genetic marker $RCIA_{39}$

Group of children	Strain characteristics in respect of the $RCIA_{39}$ marker	Percentage of strains with indicated $RCIA_{39}$ genetic characteristics in different observation years				
		1976–1977	1977–1978	1978–1979	1979–1980	1980–1981
Often diseased (34/54)	High	40.7	1.8			
	Moderate	7.4	22.2			
	Low	7.4		11.1		
	None				1.8	7.4
Moderately diseased (64/112)	High	17.8	6.25		2.7	
	Moderate	23.2	2.7	3.6	0.9	
	Low	11.6		12.5	4.5	
	None				14.3	
Rarely diseased (12/21)	High	4.8				
	Moderate	14.3		14.3		
	Low	4.8		42.8		
	None				14.3	4.8

Note: In this Table and in Table 2 the numerator indicates the number of children examined and the denominator — the number of isolates.

and the avirulent strains $0 = RCIA_{39} < 1.0$. A direct correlation has been found between the individual features of the macroorganism (different extent of general resistance to respiratory viral infections and to RS virus infection in particular), and the character of changes in biological pattern in respect of $RCIA_{39}$ marker of the virus populations isolated in different years. Thus, for instance, during the epidemic season 1976—1977 from children often afflicted by the disease high-yield viruses were isolated in 40.7% of cases, from moderately sick children in 17.8% of cases, but from those rarely afflicted by the disease in 4.8% of cases only. On the other hand, low-yield pathogens or RS strains which entirely lost their ability to reproduce in HEK cells at 39 °C mostly affected the groups of rarely diseased children (Table 1).

Time-dependent changes of natural RS virus populations with respect to the RCNI marker

The circulating populations of RS viruses were rather heterogenous in respect of the degree of their sensitivity to antibodies. $RCNI \leq 1.4$ was the attribute of strains showing low sensitivity to antibodies, moderately sensitive ones had $1.4 < RCNI \leq 2.0$ and the highly sensitive strains showed $RCNI > 2.0$.

Table 2. Specific features of host-virus interaction as related to the natural variability of RS viruses in respect of the RCNI marker

Group of children	Strain characteristics in respect of the RCNI marker	Percentage of strains with indicated RCNS genetic characteristics in different observation years		
		1976—1977	1977—1978	1978—1979
Often diseased (34/51)	Low	39.2	21.5	5.9
	Moderate	17.6	13.7	
	High			1.9
Moderately diseased (64/88)	Low	46.5	5.7	1.1
	Moderate	18.2	4.5	4.5
	High	3.4	1.1	14.8
Rarely diseased (12/16)	Low	12.5		
	Moderate	6.2		18.7
	High	6.2		56.2

The analysis of the composition of virus population indicated to an inversed relationship between the intensity of reproduction of viruses in cells at 39 °C and the degree of their sensitivity to antibodies. Low-sensitive to antibodies strains could be isolated at nearly equal frequency from children often afflicted by the disease as well as from moderately diseased children (Table 2). The frequency of their isolation was significantly reduced (to 12.5%) in the groups of rarely diseased children. In contrast, the high-reactive strains were predominantly isolated from children who were rather resistant to acute respiratory infections (Table 2).

The rate of generation changes of natural virus populations with respect to the $RCIA_{39}$ marker

The variability of the biological pattern of RS virus population with respect to $RCIA_{39}$ was continuous which is consistent with the uninterrupted course of the epidemic process and with the variability from low to high intensity. Table 3 shows the characteristics of strains with changed activity of reproduction at various times of observation. It can be seen that about 50% of the strains had an altered $RCIA_{39}$ marker already after $1^1/_2$ or 2 months from the end of disease. The intensity of the changes reached its maximum by 5 to 6 months from the end of laboratory-confirmed outbreaks of acute respiratory disease of RS virus aetiology among the nursery-school children. As a rule, this coincided with the summer period. Summer strains were characterized by low infection potential. Later on in autumn, the strains changed their ability to reproduce at elevated incubation temperature. In the course of the epidemic process, RS strains were isolated revealing different reproduction abilities; the high-yield, moderate-yield,

Table 3. Variability of natural RS virus population in respect of the $RCIA_{39}$ marker as related to the period of observation and to the category of the children examined

Group of children (No.)		The number (%) of strains changed in respect of the $RCIA_{39}$ marker	The number of strains showing changed activity of reproduction at indicated observation intervals (months)						
			<1	1.0–1.5	1.6–2.0	2.1–3.0	3.1–4.0	4.1–5.0	5.1–6.0
Often diseased (54)*	abs.	8		3	2	1	1	1	
	%	(14.8)							
Moderately diseased (112)	abs.	20	3	5	3	3	2	2	2
	%	(17.8)							
Rarely diseased (21)	abs.	2		2					
	%	(9.5)							
Total (187)	abs.	30	3	10	5	4	3	3	2
	%	(16.0)	(10.0)	(33.3)	(16.6)	(13.3)	(10.0)	(10.0)	(6.7)

* Note. In parentheses the number of virus isolates.

low-yield, and none-yield viruses were — in respect of their $RCIA_{39}$ marker — denoted as +++, ++, +, and —. The manifestation intensity of this marker among natural virus populations changed in direction: +++ → → ++ → + → —. The biological properties at different levels of reversion related to the extent of the epidemic process later on altered as follows: + → ++ → +++.

Different resistance of the children organisms to respiratory diseases seems to exert dissimilar selecting effects on selection of strains with particular biological properties. It appeared that the range of variability of virus strains in respect to the $RCIA_{39}$ marker was significantly broader in chilren often or moderately afflicted by the disease (+++ ⇄ ++ ⇄ +) than in rarely diseased children (+ → ++; ++ → —).

Discussion

It has been previously shown (Yurlova *et al.*, 1983) that circulating populations of RS viruses in different years of isolation had different $RCIA_{39}$ and RCNI genetic markers and the biological pattern of the virus populations was responsible for different intensity of development of the epidemic process among nursery-school-attending children. However, the relationship between the extent of manifestation of these markers of strain differentiation and the individual features of the macroorganism has not been studied. It is known that local variability of natural viral populations is related to both genetic and phenotypical variability of the populations and subpopulations (separate virus clones). Complicated intrapopulation relations between infectious and noninfectious virus particles finally bring about the selection of high- and low-yield clones.

Our experiment have shown that high-yield viruses were predominantly isolated from the children who were often or moderately afflicted by the disease and vice versa, the low-yield viruses from rarely diseased children. It is known that individuals with high resistance to respiratory viral infections are also characterized by high level of nonspecific protection (Nevedomskaya, 1969; Popova, 1977). We believe that the factors of nonspecific resistance (interferon and inhibitors) promote the inhibition of the reproduction of high-yield clones and the selection of low-yield clones which leads to phenotypical variability of viral populations. Our studies over many years have demonstrated that the range of natural population variability of RS viruses with respect to the intensity of genetic marker $RCIA_{39}$ changed in the following directions: $+++ \rightleftarrows ++ \rightleftarrows + \rightarrow -$. This indicates to the recycling character of natural variability of RS virus, to certain population waves responsible for the phenotypical structure of natural viral populations and rapid changing of their generation. However, further influence should be considered on the natural selection of host selecting factors, providing different biological levels of the adaptation of the pathogen to host cells.

References

Kantorovich-Prokudina, E. N., and Zhdanov, V. M. (1976): Dynamic polymorphism of viral populations (in Russian). *Vop. Virus.* **21**, 650–655.

Kovaleva, T. P. (1974): Interrelations of cytotoxic activity of adenoviruses with their interferon properties and sensitivity to antibodies (in Russian). Thesis, State Research Institute of Influenza, Leningrad.

Nevedomskaya, G. N. (1969): Biological activity of virus-neutralizing inhibitors of human serum (in Russian), pp. 15–17. In Transactions of scientific papers: *Actual Problems of Antiviral Immunity During Influenza*. Leningrad.

Popova, T. L. (1977): Epidemiological characteristics of influenza and acute respiratory disease in group of children under long-term observation (in Russian). Thesis, State Research Institute of Influenza, Leningrad.

Rokitsky, P. F. (1967): *Biologicheskaya Statistika*, pp. 114–179. University Publishers, Minsk.

Selivanov, A. A., Lysov, V. V., Yurlova, T. I., and Aksenov, O. A. (1972): Comparison of two methods of titration of adenoviruses (in Russian). *Vop. Virus.* **17**, 600–603.

Tsilinsky, Ya. Ya. (1982): Viral genetics (in Russian), pp. 213–260. In Transactions of scientific papers: *Obschaya i Chastnaya Virusologiya*, Moscow.

Yurlova, T. I., Gvozdilova, D. A., Zvereva, E. P., Korovina, G. I., Karpova, L. S., Popova, R. P., and Stolbunskaya, S. D. (1983): Some regularities in variations of respiratory syncytial virus strains during their epidemiological circulation. *Acta virol.* **27**, 251–256.