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POPULATION VARIABILITY OF SEROTYPE 3 ADENOVIRUSES CIRCULATING IN THE U.S.S.R.

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Summary. — Composition analysis of natural populations of adenovirus serotype 3 (Ad 3) strains — based on samples selected out of 170 strains isolated in different regions of the U.S.S.R. from 1976 to 1981 — has demonstrated their inhomogeneity with respect to cytopathogenicity and haemagglutination activity. The strains were classified into 3 groups: low, medium and highly cytopathic. The cytopathogenicity of the strains appeared to be a rather variable feature dependent on regional climate and on seasonal complexity. In summer low cytopathogenic viruses predominated. The epidemic activity of circulating adenoviruses (proportion of adenoviruses in the aetiology of acute respiratory disease) correlated with the level of their cytopathic activity.

Key words: adenoviruses; population; cytopathic activity; natural variability

Introduction

Broad variety of virus strains is created by the change of their antigenic and biological properties under natural conditions. Some viruses (orthomyxoviruses, reoviruses, orbiviruses, arenaviruses) possess a high, some (adenoviruses, herpesviruses, poxviruses) a moderate and others (picornaviruses, togaviruses, paramyxoviruses) a low evolution plasticity.

In recent years a tendency to changes in the virulence potential has been observed in some adenovirus serotypes. The observations on adenovirus circulation, carried out over many years in Czechoslovakia (Brůčková *et al.*, 1980) based on registration of several epidemiologic characteristics of adenoviral diseases (frequency of outbreaks, intensity of spread, frequency of pathogen isolation, etc.) revealed the increasing pathogenic potential of Ad 5. The analysis of viral DNAs by means of specific restrictases indicated that isolates from 1978—1979, though identical to each other, differed from the prototype Ad 5 strains. Changes in pathogenicity of adenovirus genomes from subtype 7 to subtype 7b in the course of epidemic circulation

have been reported. The study on the spread of Ad 7b has shown that this virus was responsible for most of the severe adenovirus infection outbreaks registered in different regions of the world during last 20 years. It should be also noted that diseases caused by Ad 7b had a generalized course with signs of damage to the CNS (Wadell *et al.*, 1980).

During the analysis of natural virus population one often comes across intermediate variants with antigenic characteristics x/y, where x was determined by neutralization, and y by haemagglutination-inhibition (HI) technique. The following variations of the antigenic formula of adenoviruses have been revealed: 14 + 21/21, 14 + 16/16, 3/7, 21/16, 7/3, 11/14, 15/9, 9/27, 20/13, 26 + 27/26, 32/27, 13 + 30/10, 21/21 + 35,/ 32/10 + 19 (Parks *et al.*, 1967; Crandell *et al.*, 1968; Hierholzer *et al.*, 1974, 1980; Hierholzer and Pumarola, 1976) indicating parallel typing of the adenovirus serotypes either with respect to their neutralization or HI [numerators or denominators, respectively, for instance, types 14 and 21 (14 + 21), etc.] Clinical course of the diseases caused by intermediate adenovirus variants may vary from light to extremely severe forms in individual patiens (Hierholzer *et. al.*, 1980).

So far, the changes of antigenic and biological properties in the course of epidemic circulation of Ad 3, which the most contribute to the development of acute respiratory disease (ARD) have been insufficiently studied. The purpose of the present paper has been to determine on the basis of separate population samples the cytotoxic pattern of Ad 3 populations, circulating in the U.S.S.R. from 1976 to 1981 as related to the seasons and climatic-geographic zones.

Materials and Methods

Viruses. Of the strains of Ad 3 submitted to the All-Union Research Institute of Influenza (Ministry of Health of the U.S.S.R.), the variants have been selected, isolated and propagated not more than 3 passages in the HeLa cells. For unification of the studies repeated indentification of the pathogens was carried out in the Institute by conventional virus neutralization techniques in HeLa cells using reference immune rabbit sera supplied by WHO. After standardization of the strains with respect to the passage level, the isolates were lyophilized and stored at −70 °C. Recovery and amplification of the strains was carried out under standard conditions in HeLa cells.

Classification of materials. All the adenovirus strains were isolated from patients with different extent of damage to the respiratory organs (from low marked damage of upper respiratory tracts to pneumonias). The strains were classified with respect to the place and time of isolation. Then parallel cytotoxicity studies of the strains isolated in the same place, e.g. in Leningrad (constant feature), during various years (observation variable) have been carried out. The analysis of the materials consisted of the study of the influence of seasons and observation periods on the determined marker followed by the study of the influence of climatic-geographic factors on the cytopathic characteristics of adenoviruses. A consecutive comparative analysis of interrelations of several variables at the same constant value has been carried out, and thereafter a correlation marker analysis at different functional or constant conditional values has been made.

Cytopathic activity of the virus was determined after preliminary equalization of infective doses of pathogens at a 10^3 LD_{50}/ml value followed by heating of virus-containing material at 56 °C for 30 min. This procedure completely abolished the infective properties of adenoviruses, their toxic activity with respect to cells leaving unchanged (Ginsberg, 1957). Twofold dilutions were prepared and 1 ml of each dilution was introduced into test tubes with HeLa cell cultures and incubated for 5 days at 36 °C and 39 °C (Ginsberg, 1957). The two temperatures were chosen as

cytotoxic activity was better manifested at higher temperature (providing a broader range for characterization of the strains), and survival rate of cells at optimal temperature. However, in this paper only cytopathic characteristics of the strains obtained at 39 °C are presented. The results of daily observations were treated according to Reed and Muench. Cytopathic activity (CA) was expressed as $\log_2 CA_{50}$. Determination of cytotoxic activity was provided by all necessary controls which allowed to rule out nonspecific effect of experimental conditions on the cells.

Regression analysis. After mathematical treatment the intensity of the development of virus cytotoxicity was expressed as regression coefficients and approximated by the least-squares technique (Selivanov *et al.*, 1971). Regression coefficient (C_r) was expressed by the formula:

$$C_r = \frac{\Sigma xy}{\Sigma x^2},$$

where x was the deviation of the averaged time of incubation (a) from each day of incubation (Πx), $x = a - \Pi x$; y was deviation of the averaged values of activity (c) from daily value of activity (bx), $y = c - bx$. This provides determination of absolute values of x and y for each observation day. Developed formula for the calculation of regression coefficients can be expressed as follows:

$$C_r = \frac{\Sigma(a - \Pi x) \cdot (c - bx)}{\Sigma(a - \Pi x)^2}.$$

Haemagglutinating activity of adenoviruses was determined using two-fold dilutions of viruses and different red blood cells (1% suspension of Wistar albino rat red blood cells, 0.7 % suspension of Grevet monkey red blood cells, 1% suspension of rat red blood cells presensibilized with antiserum to serotype 6 adenovirus). Registration was conducted using conventional techniques. Adenoviruses were identified by cross-HI on the basis of traditional technique using type-specific immune rabbit sera prepared in the D.I. Ivanovsky Institute of Virology, Moscow and horse serum to 33 prototype Ad strains supplied by WHO. The viruses were classified according to the scheme proposed by Rosen (1960).

Results

Variability of adenovirus populations with respect to cytotoxic feature at various observation periods

The population of serotype 3 adenoviruses isolated in the U.S.S.R. from 1976 to 1981 appeared to be rather heterogeneous with respect to their CA. Highly toxic strains had the C of CA higher or equal to 1.1. and lower than

Table 1. Cytopathogenicity of Ad 3 strains isolated in the U.S.S.R. from 1976 to 1981

Year	Number of strains tested	Percentage of strains with indicated level of CA		
		low	medium	high
1976	16	62.5	31.2	6.3
1977	43	48.8	39.5	11.7
1978	49	43.0	28.5	28.5
1979	30	70.0	13.3	16.6
1980	12	83.4	8.3	8.3
1981	21	81.0	9.5	9.5
Total	171	58.5	25.1	16.4

Table 2. The influence of climate and geographic peculiarities on CA of Ad 3

Climatic-geographic region	Number of strains tested	Percentage of strains with indicated level of CA		
		low	medium	high
North Russia	16	81.3	12.5	6.2
North-West Russia	3	0	100	0
Central Russia	30	73.4	13.3	13.3
South-East Ukraine	62	43.5	29.0	27.4
West Ukraine	12	75.0	16.7	8.3
Byelorossia	12	58.3	25.0	16.7
Volga region	20	65.0	30.0	5.0
Ural	6	33.3	33.3	33.3
West Siberia	5	80.0	20.0	0
Far East	5	60.0	40.0	0
Total	171	58.5	25.1	16.4

2.6, the moderate cytophatic strains higher or equal to 0.5 and lower than 1.1, and the low cytopathic strains higher or equal to zero and lower than 0.5. Maximum proportion of both medium and highly cytopathic strains was observed in 1978 — 1979. During following years a tendency to the increase in the number od low CA variants (Table 1) has developed in the population. The difference was statistically significant ($P < 0.05$).

Climatic-geographic variations of cytopathic pattern of adenoviruses

Climate and geographic peculiarities significantly influenced the cytopathic properties of natural virus populations. Thus, for instance, in some regions of the country (South-East Ukraine, Byelorussia, Volga region, Ural, Far East), the proportion of medium and high CA variants in the total Ad virus population increased (Table 2). In other regions (North Russia, Central Russia, West Siberia, etc.) the population mainly consisted of low CA strains. The Ad virus isolates differed by the degree of haemagglutination activity. Haemagglutination titre of the samples varied from 1 : 4 to 1 : 512. No correlation between cytopathic and haemagglutinating activities of viruses has been found. With respect to cross HI, all isolates tested were similar to reference Ad 3. No intermediate variants have been found.

Seasonal variability of adenovirus populations

The level of cytopathic activity of adenoviruses was season-dependent. In summer low CA strains predominated, unlike to the influenza epidemic period of seasonal rise of acute respiratory disease ($P < 0.05$). By contrast, the number of medium CA strains was decreased in summer almost by the half (Table 3). Statistical differences were within confidence limits ($P < 0.05$).

Table 3. Cytopathogenicity of the Ad 3 of strains as related to the season and epidemiological situation from 1976 to 1981

Period of the year	Number of isolated strains	Percentage of strains with indicated level of CA		
		low	medium	high
Influenza epidemic season	46	56.5	26.0	17.4
Seasonal rise of acute respiratory disease	102	58.8	26.4	14.7
Summer	23	65.2	17.4	17.4

Relationship between the CA pattern of adenovirus populations and their epidemic potential

The degree of the development of epidemic process during adenovirus infection is determined by the morbidity level, frequency of virus isolation, incidence of clinically marked and inapparent forms and proportion of the adenovirus infections among other agents causing acute respiratory disease. We have studied only one aspect of the epidemic process: the incidence of adenoviruses in various years based on serological diagnostic of the acute respiratory disease. The serological studies over 64,660 ARD patients from 1976 to 1981 have shown that the proportion of adenovirus infections varied from 4.3 to 7.6% in various years ($P < 0.05$). The index was minimal in 1980—1981. It can be suggested that the decreased adenovirus incidence in the aetiology of acute respiratory disease was related to the change of cytopathic pattern of natural virus populations owing to the predomination of low CA variants (over 80%) during the last two years (Table 1).

Discussion

The analysis of the composition of natural serotype 3 adenovirus populations has revealed specific changes of populations with respect to cytotoxic characteristics as related to climate and geographic regions. We have obtained results similar to those of the authors (Tantawi *et al.*, 1978; Trent *et al.*, 1978), who believe that the variability of biological properties of togaviruses and poxviruses is largely dependent on natural peculiarities of the territories. It appeared that the percentage of highly and medium CA strains was rather high in some regions of our country being maximal in the South East Ukraine, Byelorussia, Volga region, Ural, and Far East (i.e. in the towns located at medium latitudes of 48°—55°).

Our findings indicate that seasonal complexes produced insignificant effect on the phenotypical character of adenovirus populations, in which the proportion of low CA variants increased in summer, as compared to other seasons. It was previously demonstrated that, in contrast to adenovirus

infection, respiratory syncytial virus infection is characterized by marked seasonal variability of the virulence level of circulating strains. Thus, for instance, the population of "summer" strains consisted of low-virulent variants in 88.8% of cases (Yurlova *et al.*, 1983). It can be suggested that respiratory syncytial population undergoes to higher mutation rate, as compared to adenovirus population, although adenoviruses have a greater capacity for complementation and recombination (Tsilinsky, 1982). It should be added that respiratory syncytial viruses can reproduce by one step reproduction curve at high multiplicity of infection, which is not characteristic of adenoviruses.

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