III. PROPHYLACTICAL PROBLEMS

CHARACTERIZATION OF SOME BIOLOGICAL MARKERS AND ANTIGENIC STRUCTURE OF INFLUENZA VIRUSES TYPE A ISOLATED DURING THE PERIOD 1986–1987 IN SEVERAL DISTRICTS OF NORTH-EASTERN BULGARIA

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It is known that molecular biology and genetics of influenza vuruses develops rapidly in recent decades resulting in a series of important achievements in this field.

The problem of the origin and mechanisms of development of new epidemic influenza strains as well as of their antigenic and genetic characteristics continues to be one of the most principal and actual points of contemporary biology and epidemiology of influenza viruses. It is assumed that there is a successive and systemic changeability of the antigenic composition of supercapsular proteins of influenza viruses during their circulation in natural conditions (1-5). It is known that certain antigenic groups of the virus can alternatively prevail in dependence on the immunological profile of the population. Antigenic "drifts" and "shifts" of influenza viruses set in characterized by insignificant or manifested structural modifications and changes of surface proteins of circulating influenza viruses outlining the clinico-epidemiological and immunological findings of influenza viruses (6-11).

In the present communication we present the results from our investigations of the antigenic structure and some genetic markers of influenza viruses isolated in this region during the epidemic wave 1986-1987.

Material and Methods

The following genetic markers were studied in some of 30 influenza virus strains isolated: isolation ability on chick embryo model; hemadsorption, hemagglutination, inhibitor-sensitive, elution, pathogenic, infections, toxic, and immunogenic properties; hemagglutinin and neuraminidase thermostability; reproduction ability under different temperature conditions on chick embryo model; their immunogenic and neurominidase activity as well as their antigenic structure. The complex virological, immunologic, biologic, biochemical and biophysical methods used are previously described elsewhere.

Results and Discussion

Influenza virus type A strains isolated during epidemic outbreaks in the Varna region during the period 1986-1987 were characterized by relatively difficult isolation ability on chick embryo model (up to 3-4 passages). Investigations of their hemadsorption and hemagglutination activity at 4°C and 20°C showed their different ability to agglutinate animal (10 species) and human erythrocytes of the "O" group. Most strains manifested a well-expressed hemadsorption and hemagglutination activity at both temperatures to erythrocytes from hen, guinea pig, rat, mouse as well as to human erythrocytes of the "O" group.

Influenza strains isolated during this period possessed an outlined adsorption (30 min) and different elution activity at 37°C in physiological saline by using heated and unheated

virus. The degree of elution of strains isolated in 1986-1987 as well as of standard strains (table 1) A (Victoria) 3/75, A (Brazil) 11/78, A (Bangkock) 1/79, and A (Philipines) 2/82 presented from 1:4 till 1:64 as compared to the initial titre. The studies of hemagglutinin thermostability of epidemic influenza virus strains (for 1986-1987) demonstrated differing properties.

Table 1

Results from the study of the adsorption and elution properties of influenza viruses isolated during the influenza epidemics in 1986–1987

Influenza virus strains	Antigenic formula	Heated or unheated	Initial titre	Elution activity at 37°C for:				
				15 min	30 min	60 min	120 min	180 min
A/Vn/2/5/87	A(H3N2)	yes	1:32	1:16	1:16	1:8	1:8	1:8
		no	1:256	1:128	1:128	1:128	1:64	1:64
A/Vn/4/9/87	A(H3N2)	yes	1:64	1:32	1:32	1:16	1:8	1:8
		no	1:128	1:64	1:64	1:32	1:32	1:32
A/Vn/4/10/87	A(H1N1)	yes	1:128	1:64	1:32	1:32	1:16	1:8
		no	1:512	1:256	1:256	1:128	1:64	1:32
A/Vn/9/1/87	A(H3N2)	yes	1:256	1:128	1:64	1:32	1:16	1:4
		no	1:512	1:256	1:128	1:128	1:64	1:64
A/Vn/9/9/87	A(H1N1)	yes	1:64	1:32	1:16	1:16	1:8	1:4
		no	1:256	1:64	1:64	1:32	1:32	1:16
A/Vn/14/1/87	A(H1N1)	yes	1:64	1:32	1:32	1:16	1:8	1:8
		no	1:256	1:128	1:64	1:64	1:32	1:32
A/Victoria/3/75	A(H3N2)	yes	1:64	1:32	1:32	1:16	1:16	1:8
		no	1:256	1:128	1:128	1:64	1:64	1:32
A/Brazil/11/78	A(H1N1)	yes	1:32	1:16	1:16	1:8	1:8	1:4
		no	1:64	1:32	1:32	1:32	1:32	1:32

Some strains with manifested thermostabile and thermolabile properties of surface proteins (table 2) were outlined. They were similar to standard strains A (Bangkock) 1/79 and A (Philipines) 2/82 when this sign was concerned. Influenza virus strains studied demonstrated not only high but also low inhibitor-sensitive properties to non-specific inhibitors contained in 10 animal species and human sera. Influenza viruses showed a varying infectious, pathogenic, toxic and immunogenic activity on hen embryos and experimental animals (cocks, guinea pigs, rats and mice).

Table 2

Study of thermostabile properties of haemagglutinin of influenza viruses isolated in 1986–1987

Influenza virus strains	Antigenic formula	Initial titre	Hemagglutination titre after			
			15 min	30 min	60 min	120 min
A/Vn/2/5/87	A(H3N2)	1:128	1:4	1:4	0	0
A/Vn/4/9/87	A(H3N2)	1:256	1:32	1:16	1:16	1:16
A/Vn/4/10/87	A(H1N1)	1:256	1:256	1:256	1:128	1:128
A/Vn/9/1/87	A(H3N2)	1:256	1:16	1:8	1:4	1:4
A/Vn/9/9/87	A(H1N1)	1:128	1:16	1:8	1:8	1:8
A/Vn/14/1/87	A(H1N1)	1:256	1:256	1:256	1:64	1:64

Infectious titre of viruses studied on hen embryo model varied within the limits of $LgLD_{50} - 6.0 - LgLD_{50} = 7.5$.

These influenza viruses possessed slightly expressed toxic properties to experimental mice under conditions of intranasal and intraperitoneal infection (table 3).

Table 3

Study of the infectious and toxic properties of influenza viruses isolated during 1986–1987

Influenza virus strains	Antigenic formula	Initial titre	Number of animals	Mortality		Hemagglutin. titre of prot. susp.	Hemagglutin. titre of chick embr. inf. mouse s.	Infect. titre of chick embryos
				dead	alive			
A/Vn/2/5/87	A(H3N2)	1:512	5	1	4	1:2	1:4	10^{-6}
A/Vn/4/9/87	A(H3N2)	1:256	5	–	5	1:2	0	10^{-7}
A/Vn/4/10/87	A(H1N1)	1:256	5	1	4	1:4	1:4	10^{-7}
A/Vn/9/1/87	A(H3N2)	1:256	5	–	5	1:2	1:4	10^{-7}
A/Vn/9/9/87	A(H1N1)	1:256	5	–	5	1:2	1:4	$10^{-6.5}$
A/Vn/14/1/87	A(H1N1)	1:256	5	–	5	1:2	1:4	$10^{-6.5}$

The results from experiments for cultivation of these influenza viruses in chick embryos at 26°C, 34°C, and 40°C indicated their various reproductive activity. Most viruses could be cultivated not only under low-temperature but also under high-temperature conditions. Infectious titre of viruses cultivated at 26°C and 34oC varied between 10^{-9} and 10^{-6} but that of viruses cultivated at 40°C – between 10^{2} and 10^{3}.

The investigations of the antigenic structure of influenza virus strains isolated in 1986-1987 as well as the determination of the antigenic profile of their neurominidase component showed that they belong to influenza virus type A with antigenic formula A/H1N1 and A/H3N2.

Strains with antigenic formula A/H1N1 showed antigenic similarity to standard strains A (Brazil) 11/78, A (England) 333/80, A (Chile) 1/83, and A (Switzerland) 79/86. Influenza virus strains with antigenic formula A/H3N2 were similar to standard ones A (Texas) 1/77, A (Bangkock) 1/79, A (Philipines) 2/82, and A (Mississippi) 1/83 (table 4).

The studies of the antigenic profile of the second supercapsular component of influenza viruses, i.e. of neuraminidase by means of the method of reaction of inhibition of neraminidase activity (RINA) demonstrated strain belonging to N2. However, neuraminidase of these influenza virus strains interacted insufficiently with antisera against recombinant influenza viruses which argued for N2 heterogeneity.

Experimental treatment of virus enzyme neuraminidase with inhibitors (glutathione, L-cysteine and Zn^{2+}) revealed that inhibitory action increased to a high extent in concentration of 0.1 M.

Glutathione exerted the most powerful inhibitory action on influenza viruses A/1986-1987 (55-70 per cent) followed by L-cysteine (55-65 per cent) and Zn^{2+} (30-40 per cent).

Our investigations showed that intensive simultaneous circulation of both antigenic variants of influenza virus type A characterized by their corresponding genetic markers and antigenic properties was going on nowadays.

Table 4

Antigenic structure of influenza viruses isolated in 1986–1987

Sera	Immune sera against our influenza virus strains					
Influenza virus strains	A/Vn/2/5/87	A/Vn/4/9/87	A/Vn/4/10/87	A/Vn/9/1/87	A/Vn/9/9/87	A/Vn/14/1/87
Homologous titre	12000	3200	12800	3200	51200	12800
A/Vn/4/9/87 A(H3N2)	1/4	1/4	1/32	1/8	1/32	1/16
A/Vn/9/1/87 A/H3N2)	1/2	1/2	1/32	1/8	1/32	1/8
A/Vn/14/1/87 A(H1N1)	1/2	1/4	1/32	1/16	1/64	1/16

Sera	Immune sera against standard influenza virus strains											
Influenza virus strains	A(Texas)1/77 A(H3N2)	A(Brazil)11/78 A(H1N1)	A(Bangkock)1/79 A(H3N2)	A(Chile)1/83 A(H1N1)	A(Philipines)2/82 A(H3N2)	A(Mississipp)1/83 A(H3N2)	A(Switzerland)79/86 A(H1N1)	A(England)333/80 A(H1N1)	B(Ann Arbor) 1/86	B(Singapore) 222/79	B(USSR)10/83	C(USA)1233/47 Normal guinea pig serum
Homologous titre	800	1600	1600	6400	1600	3200	400	3200	400	1600	800	1600 0
A/Vn/4/9/87 A(H3N2)	1/64	1/16	1/16	1/16	1/64	1/32	1/4	1/32	1/8	1/32	1/16	1/128
A/Vn/9/1/87 A/H3N2)	1/64	1/32	1/16	1/8	1/32	1/32	1/4	1/32	1/8	1/32	1/8	1/32 0
A/Vn/14/1/87 A(H1N1)	1/16	1/32	1/16	1/64	1/32	1/32	1/8	1/64	1/8	1/32	1/16	1/32 0

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ХАРАКТЕРИСТИКА НЕКОТОРЫХ БИОЛОГИЧЕСКИХ МАРКЕРОВ И АНТИГЕННОЙ СТРУКТУРЫ ВИРУСА ГРИППА ТИПА А, ИЗОЛИРОВАННОГО В 1986-1987 г.г. в НЕКОТОРЫХ ОКРУГАХ СЕВЕРОВОСТОЧНОЙ БОЛГАРИИ

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РЕЗЮМЕ

Проведены вирусологические, иммунологические, эндимологические, биохимические и другие исследования части изолированных вирусов гриппа (50 штаммов) во время гриппозной эпидемии в указанном регионе в течение 1986 – 1987 г.г. Прослеживалась последовательная изменчивость антигенных свойств поверхностных белковых компонентов гриппозных вирусов, а также их биологическая характеристика.

Изучены следующие биологические свойства и генетические маркеры гриппозных вирусов: изолируемость модели куриных эмбрионов, а также их гемадсорбционные, гимаглютинационные, ингибиторчувствительные, элюирующие, патогенные, инфекционные, токсические и иммуногенные свойства. Прослеживались также термостабильность гемаглютинина и невраминидазы, их способность к репродукции на модели куриных эмбрионов при температуре 26^{o}, 34^{o}, 40^{o} С. Учитывались и их невраминидазная активность, а также их антигенная структура.

Проведен анализ вирусологической и эпидемиологической периодичности исследуемых гриппозных щтаммов.