III. Prophylactical problems

STUDY OF THE ANTIGENIC STRUCTURE, IMMUNOLOGICAL ACTIVITY AND SOME GENETIC MARKERS OF INFLUENZA VIRUSES ISOLATED IN 1987 - 1988

G. Kaprelyan, V. Rusev, M. Chividzhyan, K. Tuncheva, A. Saruyan

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Recently, molecular biology of influenza viruses advances rapidly and enables the solving of numerous essential problems concerning the nature of the virus and influenza pathogenesis and immunity.

Antigenic instability of influenza viruses, type A presents an unique peculiarity (1,3). The source and mechanisms of origin of new epidemic influenza strains are one of the actual topics of modern biology and epidemiology of influenza viruses. It is assumed that there exists a successive and systemic antigenic variability of influenza viruses during their circulation under natural conditions (2, 5, 6). It is also considered that when new influenza viruses, type A appear (characterized by new antigens) the etiological role of previously circulating influenza virus strains decreases or even disappears (4, 11)1 Antigenic changes of the structure of surface protein components of influenza viruses characterize, probably, the clinico-epidemiological and immunological findings of influenza epidemics (7, 9, 10).

In the present scientific communication we report our results from the investigations of the antigenic structure and genetic markers of influenza viruses, type A isolated in the region of Varna in the period 1987 — 1988.

Material and methods

The following biological properties and genetic markers were studied in five out of 15 isolated influenza virus strains: ability to be isolated on chick embryo model and adaptability to some cellular cultures; hemagglutination, eluation, inhibition-sensitive, pathogenic, infectious, toxic, and immunogenic properties; thermostability of hemagglutinin, neuraminidase activity under the action of the inhibitors glutathione and L-cysteine, as well as the antigenic structure of the isolated viruses. Our complex methods used have been described in detail elsewhere (10). Determination of the residual neuraminidase activity was performed after Aminoff's method (8).

Results and discussion

The isolated influenza virus strains in the period 1987 — 1988 demonstrated a difficult isolation ability (up to 3—4 passages) on model of 9—12 chick embryos. On cellular culture model «CEK», however, influenza viruses isolated up to the 4th passage could not adapt by characteristic cytopathic alterations.

The isolated influenza virus strains as well as standard ones showed various heamabsorption and hemagglutination properties towards 11 kinds of animal and human «0» group erythrocytes at 4° and 18° C. Most strains studied demonstrated a well-expressed hemabsorption and hemagglutination activity at both temperature levels towards erythrocytes from hen, rabbit, sheep, rat, mouse, tortoise as well as towards human «0» group erythrocytes.

Our experiments indicated that these influenza viruses posessed a manifested absorption and different eluation activity at 37° C in saline by using of an unheated or heated inactivated virus. Some strains were outlined to possess a poor eluation activity, namely A/Vn 1/87 and A/Vn 1/88.

We studied the inhibition-sensitive properties of the isolated influenza virus strains towards unspecific inhibitors contained in the serum of 11 kinds of animals and man. There were influenza virus strains with high and with relatively lower reactions to unspecific serum inhibitors.

Infectious titre of these viruses on chick embryo model varied within the limits of $lgLD^{50} = -2.50$ and $lgLD^{50} = -7.54$.

The isolated influenza viruses in this period possessed a weakly expressed pathogenic, infectious, toxic and immunogenic activity on chick embryos and experimental animals (cocks and mice).

The results from the cultivation of influenza viruses on chick embryos at 26°C, 34°C, and 40°C demonstrated their heterogenic reproduction activity and hemagglutinin thermostability.

Neuraminidase activity and thermosensitivity at 56°C and 60°C as well as the effect of the inhibitors L-cysteine and reduced glutathione was estimated in these strains, too. By means of the reaction of inhibition of hemagglutination it was established that these influenza virus strains pertained to antigenic subtypes N1 and N2 when their neuraminidase was concerned. Influenza viruses with an antigenic formula H3N2 were related to the standard strain A/Leningrad 360/86 (H3N2) while the strain A/Vn(1/87 (H1N1) was related to A/England/333/80 (H1N1).

Enzyme thermosensitivity indicated (fig. 1) that enzyme activity decreased by about 80—90 per cent during the first 5—10 min of the experiment at 56°C and 60° C in all the strains examined.

Under the influence of L-cysteine and reduced glutathione there was an enzyme inhibition by approximately 50 per cent in all the standard strains used. Fig. 2 showed that local strains were inhibited to an almost equal extent in comparison with standard ones under L-cysteine action. Fig. 3 demonstrated that local strains were inhibited to a greater extent in comparison with standard ones when reduced glutathione was applied. Our data showed that glutathione was a better inhibitor than L-cysteine by 8—12 per cent as compared with the controls.

The investigations of the antigenic structure of influenza virus strains isolated in the period 1987—1988 and the determination of the antigenic profile of their neuraminidase component indicated their belonging to influenza virus,

type A with an antigenic formula A(H1N1) and A(H3N2). Strains with an antigenic formula A(H1N1) were similar to a certain extent to the standard strain A/Taiwan/1/86 (H1N1) and those with an antigenic formula A(H3N2) — with the standard strains A/Leningrad /360/86 (H3N2) and A/Philipines /2/82 (H3N2) (table 1 and 2).

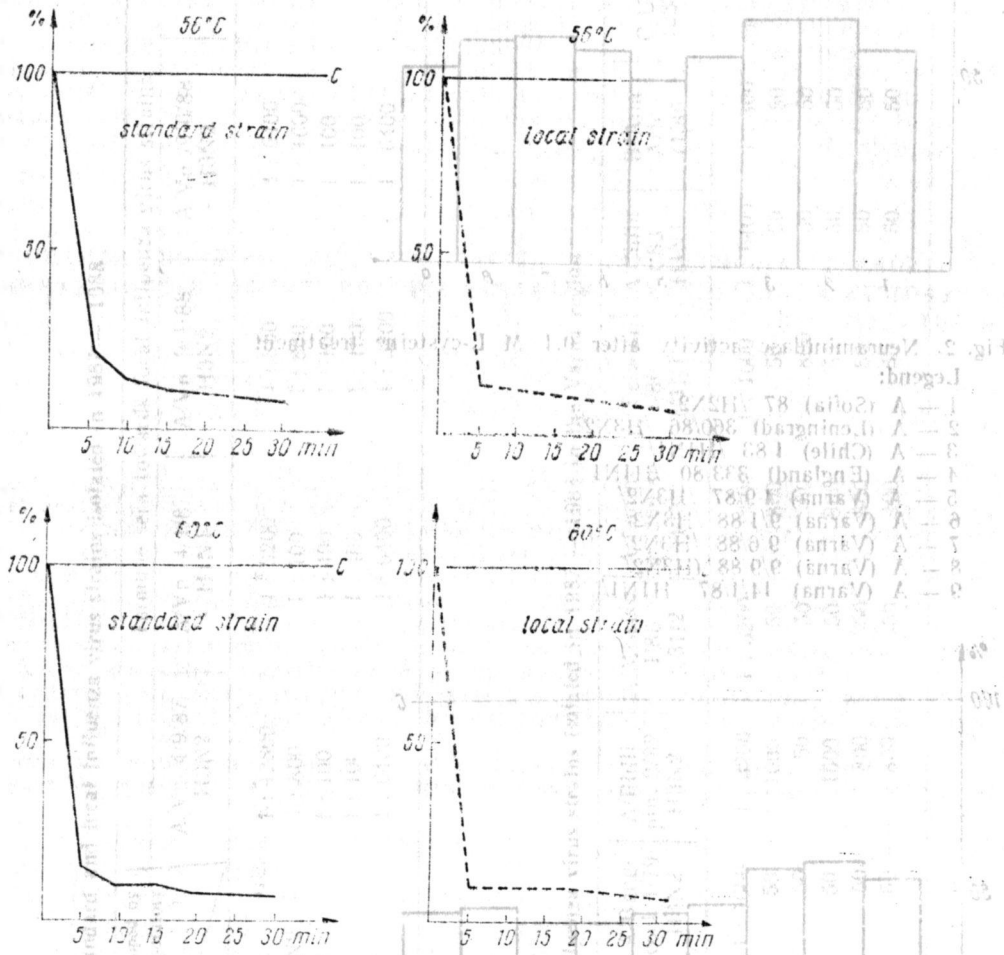

Fig. 1. Enzyme activity changes at 56°C and 60°C for different time periods.

These studies demonstrate that intensive simultaneous circulation of both antigenic variants of influenza virus type A with their corresponding genetic markers and antigenic properties goes currently on at presence.

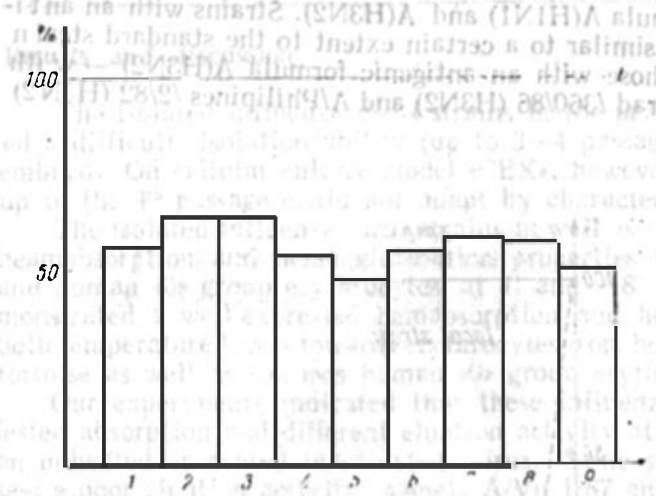

Fig. 2. Neuraminidase activity after 0.1 M L-cysteine treatment
Legend:
1 — A (Sofia) 87 /H2N2/
2 — A (Leningrad) 360/86 /H3N2/
3 — A (Chile) 1/83 /H1N1/
4 — A (England) 333/80 /H1N1
5 — A (Varna) 4/9/87 /H3N2/
6 — A (Varna) 9/1/88 /H3N2/
7 — A (Varna) 9/6/88 /H3N2/
8 — A (Varna) 9/9/88 (H3N2/
9 — A (Varna) 14/1/87 /H1N1/

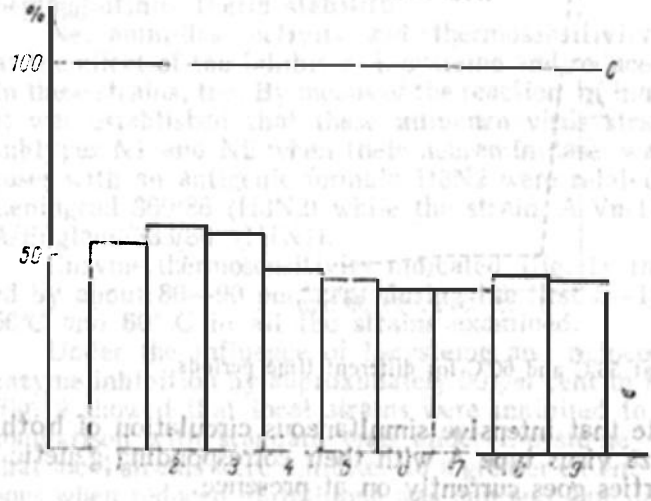

Fig. 3. Neuraminidase activity after 0.1 M glutathione treatment.
Legend — see fig. 2.

Table 1

Antigenic structure of standard and local influenza virus strains isolated in 1987 – 1988

Immune sera developed by our laboratory / Staadard strains	Immune sera towards local influenza virus strains				
	A/Vn/4/9/87 H3N2	A/Vn 14/1/87 H1N1	A/Vn 9/1/88 H3N2	A/Vn /9/6/88 H3N2	A/Vn 9/9/8 H3N2
Homologous titre	1 : 12800	1 : 3200	1 : 800	1 : 6400	1 : 12800
A/Leningrad/360/86 H3N2	1 : 800	1 : 100	1 : 800	1 : 1600	1 : 1600
A/England/333/80 H1N1	1 : 100	1 : 400	1 : 100	1 : 100	1 : 100
A/Chile/1/83 H1N1	1 : 100	1 : 400	1 : 100	1 : 100	1 : 100
A/Sofia/87 H3N2	1 : 6400	1 : 6400	1 : 3200	1 : 6400	1 : 51200

Table 2

Antigenic structure of influenza virus strains isolated in 1987 — 1988 in the Varna region

Antisera Local virus strains	A/Bang-cock 1/79 H3N2	A/Phili-pin. 2/82 H3N2	A/Mississ. 1/85 N3H2	A/Lonin-gr. 360/86 H3N2	A/Teiwan 1/86 H1N1	A/Chile 1/83 H1N1	B/Ann Arb. (1/86	C/USA 1233/47	Normal
Homologous titre	1 : 1600	1 : 3200	1 : 3200	1 : 800	1 : 1600	1 : 6400	1 : 800	1 : 6400	
A/Vn/4/9/87	50	1600	50	800	50	50	50	50	50
A/Vn/14/1/87	50	50	50	50	800	50	50	50	50
A/Vn/9/1/88	50	1600	50	800	50	50	50	50	50
A/Vn/9/6/88	50	800	50	800	50	50	50	50	50
A/Vn/9/9/88	50	800	50	400	50	50	50	50	50

REFERENCES

1. Антонова, В. И., и др. *Хрон. ВОЗ*, 33, 1979, № 4, 192—194. — 2. Голубев, Д. Б. *Итоги науки и техники, сер. Вирусология*, 185, № 11, 156 с. — 3. Закстельская, Л. Я., З. Николова, М. Яхно, и др. *Вопр. вирусол.*, 1982, № 6,671 — 676. — 4. Кебеджиев, Г., К. Кузмов, Х. Радева, и др. *Епидемиол., микробиол. и инфекц. болести*, 1981, № 1, 17—22. — 5. Коцева, Р., П. Панайотов. Л. Шиндаров. *Епидемиол., микробиол. и инфекц. болести*, 1985, № 1, 1—6. — 6. Николова, З., Р. Коцева, Г. Капрелян, и др. *Епидемиология, микробиол. и инфекц. болести*, 1985, № 2, 1—8. — 7. Шилов, А. А., Ю. В. Козлов, О. Чайка, и др. *Вопр. вирусол.*, 1984, № 4, 419—429. — 8. Aminoff, D. *Biochem. J.*, 81, 1961, 382—384. — 9. Colman, P. M., C. W. Ward. *Curr. Topics Microbiol. Immunol.*, 114, 1985, 177—255. — 10. Kaprelyan, G., L. Karaivanova, K. Metodiev, et al. In: Internat. Congr. Infect. Parasit. Dis., IX. Munich, 20—22. VI. 1986. Abstracts, p. 469; Poster, V—P—12. — 11. Webster, R. G., L. E. Brown, W. G. Zaver. *Virology*, 135, 1984, No 1, 30—42.

ИССЛЕДОВАНИЕ АНТИГЕННОЙ СТРУКТУРЫ, ИММУНОЛОГИЧЕСКОЙ АКТИВНОСТИ И НЕКОТОРЫХ ГЕНЕТИЧЕСКИХ МАРКЕРОВ ВИРУСОВ ГРИППА, ИЗОЛИРОВАННЫХ В 1987 — 1988 Г.

Г. Капрелян, В. Русев, М. Чивиджиян, К. Тунчева, А. Сарыян

РЕЗЮМЕ

С помощью современных вирусологических, биологических и биохимических методов исследовались антигенная структура, некоторые биологические и иммунологические свойства гриппозного вируса, изолированного в 1987—1988 г. в Варненской области.

Прослеживалось изменение антигенных свойств поверхностных белковых компонентов — гемаглугинина и невраминидазы двадцати штаммов вирусов гриппа типа А с антигенной формулой A/H1N1 и A/H3N2. Были изучены следующие биологические свойства изолированных вирусов гриппа: способность изолируемости модели куриных эмбрионов, а также гемадсорбционные, гемаглютинационные, ингибиторчувствительные, элюирующие, патогенные, инфекционные, токсические и иммуногенные свойства. Изучалась также термостабильность поверхностных антигенов и активность невраминидазы. Была установлена способность к репродукции изолированных штаммов вирусов гриппа при температура 26°С. 34°С и 40°С.

С помощью проведенных исследований были установлены закономерности, которые дают более полную характеристику циркулирующих в последнее время вирусов гриппа,