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HETEROGENEITY OF INFLUENZA B VIRUS STRAINS ISOLATED IN A LOCAL AREA OF THE DISEASE DURING AN INFLUENZA OUTBREAK

F. N. REIZIN, M. P. CHUMAKOV, L. I. MARTYANOVA

The USSR AMS Institute of Poliomyelitis and Viral Encephalitides, Moscow, U.S.S.R.

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Summary. — Ten strains of influenza B virus isolated in a local focus during an influenza outbreak were found to include 9 virus strain variants as demonstrated by different antigenicity of their haemagglutinin, ts-marker, sensitivity to heating at 56 °C/30 min, and to non-specific serum inhibitors. These strains induced antibodies in rats which interacted more actively with the virus isolated in earlier periods of this outbreak than with that isolated later. It might indicate that all strains originated from the same parent strain of virus, which induced the influenza outbreak in this area.

Key words: *influenza B virus*; *isolates*; *heterogeneity*

Introduction

The influenza B virus is considered to show less antigenic variabilities as compared to virus A (Schild *et al.*, 1973; Webster and Berton, 1981). With the use of monoclonal antibody it was possible, however, to demonstrate antigenic variations between influenza B virus strains that were recovered in different years, as well as during the same influenza outbreak (Six *et al.*, 1981; Lu Bao-Lan *et al.*, 1983; Oxford *et al.*, 1983). We isolated 9 strains of influenza B virus during an oubreak of the disease in a local area (Reizin *et al.*, 1985) and investigated them for antigenic and some other variations. The results are presented in this paper.

Materials and Methods

Virus strains. In addition to the above mentioned strains of influenza B virus the reference strains B/Hongkong/8/73 (B/HK), B/Singapore/222/79 B (Sing) and B/Leningrad/489/80 (B/Len) were used, for comparison, they were received from L. A. Tarasevich State Institute for standardization and Control and the All-Union Research Institute of Influenza of the U.S.S.R. Ministry of Health. The strain B/2466 obtained from the Municipal SES of Moscow, which was isolated during the same period in Moscow, was also used. All strains were passaged in the allantoic cavity of 10-day-old chick embryos (CE).

Immune sera were produced in white non-bred rats immunized without adjuvant in three doses, at days 0, 14, and 21 using a virus which underwent at least 2 passages in CE after its original isolation. The rats received different virus strains in similar doses.

Table 1. Cross-antigenicity of haemagglutinin from prototype and recently isolated strains of influenza B virus

Virus strain	HI titre*										
	B/Sing.	B/Len.	B/HK	B/473	B/476	B/480	B/487	B/488	B/496	B/498	B/2466
B/Sing./222/79	1102	1	1/2	1	1/1.5	1/2	1	1	1	1	1/1.5
B/Len./489/80	1/2	746	1/2	1	1	1/1.5	1	1/8	1	1	1/2
B/HK/8/73	1/8	1/3	5120	1/5	1/12	1/70	1/2	1/8	1/64	1/40	1/12
B/473	1/3	1/2	1/32	214	1/1.5	1/2	1	1/2	1/16	1	1/2
B/474	1/2	1/1.5	1/8	1	1	1	1	1	1/2	1	1
B/476	1/3	1/1.5	1/16	1	240	1	2	4	2	2	1/3
B/480	1/5	1/3	1/256	1/2	1	373	1/2	2	1	1/2	1/2
B/487	1/7	1/6	1/64	1/3.5	1/6	1/4	1280	4	1/2	1	1/3
B/488	1/5	1/4	1/16	1/3.5	1/4	1/4	1	1280	1/2	1/32	1/2
B/496	1/6	1/4	1/32	1/2	1/5	1/3	1	1	2560	1/16	1/5
B/497	1/3	1/3	1/16	1/2	1/2	1/2	1/2	1/8	1	1/16	1/5
B/498	1/7	1/6	1/32	1/3	1/2	1/3	1	1/32	1/40	1280	1/2
B/2466	1/6	1/3	1/32	1	1/1.5	1/1.5	1	1/2	1/2	1/32	120

* Antibody titre with heterogenous strains is expressed as the ratio of its titre with the homologous virus strain.

Table 2. Ability of influenza B virus strains to growth in chick embryos of different temperatures

Virus strain	Reciprocal HA titre at temperatures:			
	35 °C	37.2 °C	37.8 °C	38.5 °C
B/Sing./222/79	1280	1280	1280	—
B/Len./489/80	1280	1280	80	—
B/HK/8/73	1280	640	160	—
B/473	640	80	—	—
B/474	2560	20	—	—
B/476	640	320	—	—
B/480	640	80	—	—
B/487	1280	—	—	—
B/488	1280	640	—	—
B/496	1280	320	40	—
B/497	1280	160	320	—
B/498	640	40	80	—
B/2466	1280	640	80	—

Note — Titre in HA < 10.
The multiplication of infection was in the range of 0.6—1.2 HAE/CE with all strains.

Haemagglutination-inhibition (*HI*) test was made on microtitre plates according to Takátsi. *The ts-marker* of these strains was investigated in CE at 35 °C and at the tested temperature. Stability of virus haemagglutinin (HA) was determined in HA test with 1 ml virus aliquots, which were heated at 56 °C/60 min in a water bath and then cooled.

Interferonogenic activity of the virus strain was tested in CE cells (Reizin *et al.*, 1975).

Results

According to the degree of their antigenic relationship to influenza virus B/Sing, all isolated strains were divided into 2 groups (Table 1). 1. Strains B/473, B/474, B/476, B/497 which reacted relatively well with the immune-serum to B/Sing (1/2-1/3), and 2. strains B/480, B/487, B/488, B/496, B/498 that showed significantly lower interaction with this serum (1/5—1/7). All the tested strains of influenza B virus were less sensitive to antibody against influenza virus B/HK. Antibodies to the majority of these isolates completely or almost completely inhibited HA of B/Sing and B/Len. The influenza virus B/HK showed some sensitivity only to antibody against B/487.

Nearly all fresh influenza B virus isolates induced antibodies which reacted with the more recent virus isolates less actively than with those recovered in earlier periods (Table 1). However, antibody to B/487 strain interacted equally with all tested influenza B virus strains. Immune serum against B/498 showed lower interaction with the strains B/488 and B/496 that had been isolated earlier, and with B/497 strain isolated from the same specimen as B/498. As seen from Table 1, the mentioned isolates of the influenza B viruses demonstrated some differences in their immunogenic activity.

Table 3. Termostability of haemagglutinin of influenza B virus strains at 56 °C/60 min.

Virus strain	Reciprocal HA titre		
	before heating	after heating	residual activity (%)
B/Sing./222/79	640	40	6.2
B/Len./489/80	1280	<10	0
B/HK/8/73	640	640	100
B/473	320	<10	0
B/474	1280	1280	100
B/476	160	40	25
B/480	1280	160	12.5
B/487	640	640	100
B/488	640	640	100
B/496	640	20	3.1
B/497	1280	320	25
B/498	640	1280	200
B/2466	1280	320	25

Virus strains B/487 and B/474 are completely or almost completely unable to grow in CE at 37.2 °C. The inability of new isolates to replicate is increased at 37.8 °C. None of the investigated strains was able to grow in CE at 38.5 °C; no HA was found in the allantoic fluid of CE infected with these strains (Table 2). The ts-characteristics of B/487 and B/496 strains were almost unchanged by additional passages in CE at optimal temperature.

Despite of their ts-marker, the investigated strains of influenza B virus induced immune responses in guinea pigs, the normal body temperature of which is over 39 °C (Festing, 1976). According to the data reported else-

Table 4. Sensitivity of influenza B virus strains to non-specific inhibitors of normal rat serum

Virus strain	Reciprocal titre in HI with sera	
	native	heated (56 °C/30 min)
B/Sing./222/79	—	0
B/Len./489/80	—	—
B/473	1280	20
B/474	—	—
B/476	640	160
B/480	40	—
B/487	—	—
B/488	—	—
B/496	1280	80
B/497	—	—
B/498	10	—
B/2466	—	—

Note — Titre in HI < 10

shere (Othawara *et al.*, 1985), ts_{39}-strains of rubella virus were not immunogenic in rabbits that have their body temperature over 39.5 °C.

According to their HA stability at 56 °C/60 min, all tested strains of influenza B virus were divided into 3 groups (Table 3): thermostabile (B/HK, B/474, B/487, B/488), thermolabile (B/Sing, B/Len, B/473, B/476, B/480, B/496, B/497, B/2466) and the B/498 strain which HA titre increased by heating.

Three variation groups were also found according to the sensitivity of examined strains to non-specific inhibitors of normal rat serum (Table 4): non-sensitive (B/Sing, B/Len, B/HK, B/474, B/487, B/488, B/497, B/2466); sensitive to inhibitors of native, but not of heated serum (B/480, B/498) and sensitive to inhibitors of native and heated (56 °C/30 min) serum (B/476, B/473, and B/496).

All tested strains had similar interferonogenic activity.

Discussion

The data presented here demonstrate the heterogeneity of influenza B virus strains which were isolated during an influenza outbreak in a local area. With the exception of completely similar strains B/473 and B/476, the rest of them differed one from another in some characteristics, i.e. 9 of 10 isolates had strain variations.

Some investigators reported that co-circulation of different variants of influenza B virus was possible at the same time and in the same area. There are evidences of simultaneous isolation of two (Webster and Berton, 1981), eight (La Montagne, 1980), and even 14 (Oxford *et al.*, 1983) antigenically different groups of influenza B virus. As it can be seen from this paper, the investigation of biological properties, other than antigenic, reveals even more different strain variations of the influenza virus.

HA of newly isolated strains differed from those of B/Sing asymmetrically: the strains were less sensitive to antibody against B/Sing, but the latter was completely sensitive to antibody against these isolates. Antigenic variations between these new isolates were also one-sided: they showed a lower HA sensitivity to antibody against earlier isolates and were completely enough sensitive to serum antibody against the recent isolates. It might point out that all these isolates originate from the same parent strain, which induced the influenza outbreak in this area.

Heterogeneity of the tested influenza B virus strains seems to be explained by a sporadic point-mutation and by mutant selection that occurred during the outbreak. However, in one patient we also observed the excretion of different virus mutants (strains B/497 and B/498). Predominantly, mutations concerned HA gene (antigenic heterogeneity, its sensitivity to heating at 56 °C and to non-specific inhibitors). Variability of the isolates in the ts-marker indicates, however, that this mutation can occur also in other genes (Klimov *et al.*, 1984).

It will be noted that variability of the isolated strains didn't influence the clinical signs of disease. In all patients the disease was characterized by

approximately similar duration and intensity of febrile reactions, by the rate of disease manifestations and by catarrhal symptoms. On the other hand, the identical immunogenicity of the mentioned variants in guinea pigs which had high body temperature, evidenced that heterogeneity and ts-marker had no effect on the antigenic properties of the virus. Therefore, the drift of antigenic and other characteristics of influenza virus seems to have no effect on the response of a macroorganism to virus infection, at least not during one disease outbreak.

Our results indicate the necessity to study the problem of selection of optimal candidates from influenza B strain variants to be included in the polytype influenza vaccines.

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