Acta virol. **27** : 377—384, 1983

COMPARATIVE STUDIES ON INFLUENZA B VIRUS. I. BIOLOGICAL PROPERTIES AND POLYPEPTIDE COMPOSITION OF STRAINS ISOLATED IN DIFFERENT YEARS

N. L. KORCHANOVA, V. K. SIMANOVSKAYA, A. A. SOMININA, M. A. GORDON, T. N. GOLOVESHKINA, E. V. KUZNETSOVA

The All-Union Research Institute of Influenza of the U.S.S.R. Ministry of Public Health, 197022 Leningrad, U.S.S.R.

Received October 15, 1980; revised June 4, 1982

Summary. — Influenza B viruses isolated in 1940, 1950 and 1970 belonged to antigenically remote subgroups according to the antigenic composition of haemagglutinin (HA). Antigenically related strains isolated in 1972—1976 differed by their reproduction capability, by their sensitivity to inhibitors in mammalian sera and by affinity to specific antibodies in human sera. Certain correlation between these properties was observed by strains of the Hong Kong variant. Polyacrylamide gel electrophoresis (PAGE) showed that the viruses under study differed in the mobility of the major HA chain (HA_1). Molecular weights (MW) of HA_1 of antigenically similar influenza B viruses isolated in 1972—1976 revealing different biological properties varied from 52.5 K to 58 K. Neuraminidase (NA) of influenza B/Leningrad/369/75 virus purified by electrophoresis in acetate cellulose consisted of 2 polypeptides with apparent MW of 62 K and 57 K. All viruses examined showed similar mobility of the NA polypeptide MW 57 K; the mobility of the former polypeptide was similar to that of nucleoprotein (NP).

Key words: influenza B virus; biological and antigenic properties; polypeptides

Introduction

Several questions concerning biological and antigenic variability of influenza B viruses are unsolved. The influenza B viruses have been considered to represent a continuous series, slightly differing in the composition of their haemagglutinating and complement-fixing (CF) antigens (Hennessy *et al.*, 1965; Chakraverty, 1971; Luzyanina *et al.*, 1979) and sharing a common NA (Chakraverty, 1972). However, the emergence and circulation of influenza B virus strains of the Hong Kong variety, the results of their laboratory analysis and determinations of the immunological status of human population with regard to these strains indicate significant antigenic changes as

compared to influenza B viruses isolated in the fourties and fifties (Editorial Influenza, 1973*a*, *b*; 1974; Fukumi, 1973; Schild *et al.*, 1973; Seto *et al.*, 1975; Popov *et al.*, 1976, 1977). Antigenically related influenza B virus strains isolated during a single epidemic cycle may differ in a number of biological properties: reproduction capacity, sensitivity to inhibitors and affinity to antibodies (Neklyudova *et al.*, 1975; Nevedomskaya and Kudryavtseva, 1976; Popov *et al.*, 1976; Galitarov *et al.*, 1978; Yatel, 1978).

There are no definite data on the association of biological properties of influenza B viruses with their protein structure. There are some reports on polypeptide composition of the influenza B viruses, however, these were done on limited amount of antigenically remote strains (Oxford, 1973; Tobita and Kilbourne, 1975; Ivanova *et al.*, 1978). In the present study, comparative analysis of biological and antigenic properties and polypeptide composition of influenza B viruses isolated in different years was performed to elucidate their possible correlation.

Materials and Methods

Viruses. The following strains of influenza type B virus were used: B/Lee/40, B/Moskva/95/59 (B/Msk/59), B/Hong Kong/5/72 (B/HK/72), B/Leningrad/232/74 (B/Len/74), B/Leningrad/369/75 (B/Len/75), B/Leningrad/14/76 (B/Len/76), B/Khabarovsk/46/76 (B/Khab/76), B/Murmansk/2/76 (B/Mur/76), B/Sverdlovsk/1/76 (B/Sv/76). All the strains were obtained from the collection of the Laboratory of Influenza Etiology of this Institute. The viruses were propagated in the allantoic cavity of 11-day-old chick embryos (CE) inoculated in a dose of 10^3-10^4 egg infectious doses $(EID)_{50}$/0.2 ml and incubated for 72 hr at 32 °C.

The infectious activity was assayed in 11-day-old chick embryos and calculated by the method of Reed and Muench (1938).

Haemagglutination and haemagglutination-inhibition (HI) tests were performed in accordance with the recommendations of WHO (Committee on Standard Serological Procedures in Influenza Studies, 1950).

Sera. Sera from control animals (horse, rat, calves, guinea pigs, rabbits, mice) were used to study the sensitivity of virus to inhibitors. Human sera were collected from clinically normal donors in Leningrad, Vilnius, Sverdlovsk, and Odessa in 1976—1978. To remove thermolabile inhibitors, all sera were heated in a water bath at 56 °C for 30 min. Hyperimmune strain-specific sera were produced by 5 intraperitoneal injections of white rats with virus-containing allantoic fluid. Normalized serum titres were calculated according to Fazekas de St. Groth (1969), the degree of antigenic relatedness was quantitated by the method of Golubev *et al.* (1975), Paramonova and Kamforin (1975) and Paramonova *et al.* (1978). The viruses were considered to be closely related if the sum of their normalized titres was below 2.2, and to be remotely related if the sum was above 2.2. The viruses were considered to be symmetrical if the difference of the normalized titres did not exceed 0.3 and asymmetrical if it was above this value.

The significance of differences in geometric mean titres (GMT) of antibodies calculated by the method of Voroshilova *et al.* (1964) was estimated by the T-criterion in computer "Minsk-22" in the Laboratory of General Epidemiology and Cybernetics of this Institute.

Virus purification. After concentration (40 000 × g, 1 hr) the virus-containing allantoic fluid was purified through two successive sucrose gradients according to the method of Tobita and Kilbourne (1975).

Polyacrylamide gel electrophoresis (*PAGE*) of virus proteins was performed by the method of Laemmli (1970). Slab gels were formed between two glass plates (gel thickness 1.5 mm, the length of the separating gel 100—140 mm), and electrophoresis was run in an apparatus described by Studier (1973). Electrophoresis in cylindrical gels (6 × 120 mm) was performed in an apparatus of "Bücher" Co. The samples were heated to 100 °C for 2 min in a solution containing 2% sodium dodecylsulphate (SDS), 1 mol/l urea, and 0.06 mol/l Tris-HCl, pH 6.7. In

PAGE under reducing conditions, the buffer for samples contained also 0.1 mol/l dithiotreitol (DTT) and 2% β-mercaptoethanol. The separating gel contained 10% acrylamide, 0.24% methylene bisacrylamide, 0.5 mol/l urea, 0.2% SDS, 0.68 mol/l Tris-HCl (pH 8.8—9.0), 0.025% N,N,N,N-tetramethylethylene diamine (TEMED), and 0.09% ammonium persulphate. The concentrating gel contained 2.6% acrylamide, 0.46% methylenbisacrylamide, 0.1% SDS, 0.5 mol/l urea, and 0.125 mol/l Tris-HCl (pH 6.7). Gels were stained with Coomassie blue R-250 by the method of Fairbenks *et al.* (1971). The results were recorded by densitometry of stained gels in a chromoscan (Joyce-Loeble Co.) as well as by photography in the passing light.

MW of polypeptides were determined by comparison with the mobility of following protein markers (Weber and Osborn, 1969): bovine albumin (MW 66 K), egg albumin (44 K), trypsin (24 K) and RNase (14 K).

NA was purified by electrophoresis of SDS-disintegrated virus on acetate cellulose strips. A final concentration of SDS was 2%, the strips were 145 × 25.5 mm in size. Electrophoresis was performed in a "Mikrophor" chamber (Boskamp) for 1.5 hr at a voltage of 8 V/cm. The protein fraction moving to the cathode was eluted with distilled water. After removal of SDS, protein was precipitated with three volumes of acetone and dissolved in phosphate buffered saline (PBS, pH 7.2) using 0.01% sodium azide (Laver and Valentine, 1969).

Reagents. Acrylamide and N,N-methylene bisacrylamide (Reanal, Hungary), β-mercaptoethanol, dithiotreitol, urea (Calbiochem. U.S.A.), SDS, Tris, urea (Reakhim, U.S.S.R.) triple recrystallized were used.

Results

Antigenic relatedness

Comparative study by HI test of antigenic relationships between influenza B virus isolated in 1940 and 1959 and those isolated in 1972—1976 showed that the latter were antigenically remote; the sum of normalized HI titres in 3—5 repeated experiments was over 2.2. At the same time, all viruses isolated in 1972—1976 were antigenically more closely related. The sum of their normalized titres did not exceed 1.1 which allows to classify them into a single antigenic subgroup (Table 1).

Among the viruses isolated in 1974—76 certain differences in symmetry were noted. They were all symmetrically related to the B/HK/72 virus; however, some strains were asymmetrical as related to each other, the difference in their normalized titres ranging over 0.3 (for instance, strain pairs B/Len/74 and B/Len/75, B/Len/74 and B/Mur/76, B/Len/76 and B/Khab/76, B/Len/76 and B/Mur/76). The rest of virus pairs retained the marker of antigenic symmetry.

Reproduction capability

The isolated influenza B viruses differed significantly in accumulation of HA and infectious virus in CE. B/Len/75 and B/Len/76 viruses proved to be high yielding (haemagglutination titres of 256 to 1024, infectious titres 7.5—9.0 log EID_{50}/0.2 ml), while the B/HK/72 and B/Sv/76 viruses were low yielding (haemagglutination titres 16 to 128, infectious titres 5.0—7.1 log EID_{50}/0.2 ml); the rest of strains occupied an intermediate position.

Sensitivity to inhibitors

All virus strains were found to be resistant to native and heated (56 °C, 30 min) inhibitors in horse and rat sera. As for the sensitivity to non-specific

Table 1. Normalized titres of influenza B viruses in HI cross-tests

Antisera	Virus strains								
	B/HK/72	B/Len/74	B/Len/75	B/Len/76	B/Sv/76	B/Khab/76	B/Mur/76	B/Msk/59	B/Lee/40
B/HK/72	0.00	0.54	0.2	0.2	0.58	0.36	0.2	2.2	2.8
B/Len/74	0.36	0.00	1.0	0.0	0.72	0.0	1.2	2.38	1.5
B/Len/75	0.22	0.3	0.0	0.08	0.80	0.9	1.0	2.38	1.5
B/Len/76	0.5	0.3	0.0	0.0	0.17	0.5	1.0	1.38	1.5
B/Sv/76	0.5	0.6	0.81	0.03	0.0	0.0	0.5	2.38	1.5
B/Khab/76	0.26	0.0	0.68	0.14	0.13	0.0	0.6	2.38	0.9
B/Mur/76	0.0	0.0	1.11	0.61	0.21	0.43	0.0	n.d.	1.5
B/Msk/59	2.07	1.5	1.5	1.38	1.1	2.38	n.d.	0.0	2.38
B/Lee/40	2.07	1.5	1.5	1.5	2.07	1.33	1.5	2.38	0.0

n. d. = not done

Table 2. Apparent MW of HA_1 of influenza B viruses isolated in different years

Viruses	Isolation year	MW HA_1
B/Len/76	1976	58.0*
B/Len/75	1975	55.5
B/Mur/76	1976	53.5
B/Khab/76	1976	53.5
B/Len/74	1974	53.5
B/Sv/76	1976	53.0
B/HK/72	1972	52.5
B/Msk/59	1959	57.5
B/Lee/40	1940	56.0

* in kilodaltons

inhibitors in heated and native mouse, calf, guinea pig and rabbit sera, all strains could be divided into 3 groups. B/Len/76 (inhibitor titres 40 to 2560), B/Len/74, B/Len/75, and B/Msk/59 (40 to 320) were the most sensitive to serum inhibitors in HI tests. In contrast, strains B/HK/72 and B/Sv/76 (titres from 10 to 160) were at least sensitive. The rest (B/Lee/40, B/Mur/76, B/Khab/76) belonged to the intermediate group of moderately sensitive viruses which were resistant to inhibitors of native and heated mouse and calf sera but relatively sensitive to inhibitors of native (80) and heated (10—40) guinea pig and rabbit sera.

Reactivity with human serum antibodies

Comparative studies on reactivity of influenza B viruses in question for detection of antibodies in human sera by means of HI test were carried out in 6 experiments with 314 serum specimens from adult donors. The influenza B/Len/75 virus was included as reference strain. The antigenically closely related viruses isolated in 1972—1976 were found to differ markedly in their reactivity with specific antibodies when tested with human sera in contrast to rat hyperimmune sera.

B/HK/72, B/Sv/76, and B/Mur/76 viruses showed the lowest reactivity in HI tests. GMT of antibodies determined with these viruses ranged from 0.5 to 3.15 and was significantly lower ($P = 99.9$) than that obtained with the other viruses isolated in recent years. The strains B/Len/74, B/Len/75, and B/Len/76 reacted best with antibodies in human sera obtained from different regions of the country (GMT 22.6, 10.6 to 30.0, and 15.9 to 42, respectively; the difference between GMT values was insignificant — $P<95$).

Polypeptide analysis

To compare the mobility of individual polypeptides, all 9 virus strains were subjected to PAGE (Fig. 1). With the exception of the HA_1 chain, the appropriate polypeptides of different viruses had the same electrophor-

etic mobility. Thus, the polypeptides of different viruses had similar apparent MW. The MW of NP was 62 K, that of two minor components 44 K and 36 K. The B/HK/72 and B/Sv/76 viruses had the lowest MW of HA_1 (Table 2) equal to 52.5 K and 53 K, respectively. Among viruses isolated in 1974—1976 the highest MW of HA_1 was found in B/Len/76 and B/Len/75 viruses (58 K and 55.5 K). The differences in MW of uncleaved HA synthesized under non-permissive conditions corresponded to values obtained for HA_1 and were not associated with virus isolation chronology.

In order to determine the MW of NA and identify them in electrophoregrams, NA was isolated from the influenza B/Len/75 virus. In SDS-PAGE, the NA was separated into 2 polypeptides with MW 62 K and 57 K (Fig. 2), with the latter predominating. The mobility of polypeptide MW 62 K coincided with that of NP in the whole B/Len/75 virion. NA polypeptide of MW 57 K could be seen in electrophoregrams of whole virions with the exception of cases where the MW of NA and HA_1 coincided.

Discussion

The study of antigenic relationships among influenza B virus using the criteria of relatedness and symmetry from Fazekas de St. Groth (1969) and Golubev *et al.* (1975) showed that strains isolated in recent years were antigenically related to influenza B/Hong Kong/5/72 virus but differed from viruses isolated between 1940—1950. Intragroup antigenic differences among viruses circulating in 1972—1976 were manifested in the appearance of asymmetrical variants (with retention of symmetry to the progenitor of the epidemic cycle, B/Hong Kong/5/72) which attested to differences in their age hierarchy (Fazekas de St. Groth, 1969).

According to other biological properties (reproduction capability, resistance to inhibitors in mammalian sera, reactivity with antibodies in human sera), influenza B virus strains could be facultatively divided into 3 groups. The influenza B/HK/72 and B/Sv/76 strains comprised group I (viruses of low reactivity, resistant to inhibitors and poorly reproductive). Group III showed markedly polar properties and included influenza B/Len/75 and B/Len/76 viruses. Correlation of the biological properties of these viruses with their polypeptides showed that viruses of group I had the HA_1 polypeptide of lowest MW (52 K—53 K), whereas strain B/Len/76 belonging to group III (high reproduction and antibody reactivity) had HA_1 of the highest MW (58 K).

NA isolated from the B/Len/75 strain could be separated under permissive conditions into 2 polypeptides of apparent MW 57 K and 62 K which corresponds to the results of Kilbourne *et al.* (1972) who also obtained 2 NA polypeptides. Simultaneous electrophoresis of B/Len/75 with other viruses under study showed all of them to contain a polypeptide with MW 57 K, which suggested no changes in the MW of NA polypeptide of influenza B viruses isolated in different years.

References

Chakraverty, P. (1971): Antigenic relationships between influenza B viruses. *Bull. Wld Hlth Org.* **45**, 755–766.

Chakraverty, P. (1972): Antigenic relationships between the neuraminidases of influenza B virus. *Bull. Wld Hlth Org.* **46**, 473–476.

Committee on standard serological procedures in influenza studies (1950): An agglutination-inhibition test proposed as a standard of reference in influenza diagnostic studies. *J. Immunol.* **65**, 347–353.

Editorial: Influenza B (1973*a*): *Brit. med. J.* **4**, 559.

Editorial: Influenza in the world, October 1972–March 1973. (1973*b*): *Wkly epidem. Rec.* **48**, 449–460.

Editorial: Influenza B (1974): *Vop. Virus.* (in Russian) **19**, 105–106.

Fairbenks, G., Steck, T. L., and Wallach, D. F. H. (1971): Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry* **10**, 2606.

Fazekas de St. Groth, S. (1969): New criteria for the selection of influenza vaccines. *Bull. Wld Hlth Org.* **41**, 651–657.

Fukumi, H. (1973): New B type influenza. *Clin. Virol.* **1**, 211–216.

Galitarov, S. S., Reikh, L. Ya., and Volosova, V. L. (1978): The results of the study of the antigenic structure and some biological properties of influenza B viruses isolated in the autumn-winter period of 1976–1977 (in Russian). *Problemy Grippa i ORZ* **21**, 8–13.

Golubev, D. V., Paramonova, M. S., Medvedeva, M. N., and Poljakov, Yu. M. (1975): Influenza A2 neuraminidase and its changes during persistence of viruses in tissue cultures. *Z. ges. Hyg.* **4**, 324–329.

Hennessy, A. V., Minuse, E., and Davenport, F. M. (1965): A twenty-one year experience with antigenic variation among influenza B virus. *J. Immunol.* **94**, 301–321.

Ivanova, V. T., Zakstelskaya, L. Ya., Shenderovich, S. F., Yakhno, M. A., and Zhdanov, V. M. (1978): Electrophoretic mobility of polypeptides in different human influenza A and B viruses (in Russian). *Vop. Virus.* **23**, 287–291.

Kilbourne, E. D., Choppin, P. W., Schulze, I. T., Scholtissek, C., and Bucher, D. L. (1972): Influenza virus polypeptides and antigens. Summary of influenza workshop I. *J. infect. Dis.* **125**, 447–455.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* (*Lond.*) **227**, 680–685.

Laver, G. W., and Valentine, R. C. (1969): Morphology of the isolated hemagglutinin and neuraminidase subunits of influenza virus. *Virology* **38**, 105–119.

Luzyanina, T. Ya., Nevedomskaya, G. N., Taros., L. Yu., Kudryavtseva, V. K., and Smorodintsev, A. A. (1979): Antigenic relationships between strains of influenza B virus. *Acta virol.* **23**, 113–119.

Neklyudova, L. I., Fedorova, Yu. B., Gummenik, A. E., and Orlova, N. G. (1975): Comparative study of the properties of influenza B viruses isolated in 1959–1974 (in Russian). *Vop. Virus.* **20**, 458–463.

Nevedomskaya, G. N., and Kudryavtseva, V. K. (1976): Study of the antigenic properties of influenza B viruses isolated in Leningrad, 1974 (in Russian). *Problemy Grippa i ORZ* **14**, 40–44.

Oxford, J. S. (1973): Polypeptide composition of influenza B viruses and enzymes associated with the purified virus particles. *J. Virol.* **12**, 827–835.

Paramonova, M. S., and Kamforin, L. E. (1975): Criteria of Fazekas de St. Groth in the study of influenza type A virus strains of the post-Hong Kong variety (in Russian). *Problemy Grippa i ORZ* **12**, 17–22.

Paramonova, M. S., Belyaeva, T. A., and Golubev, D. B. (1978): On mathematical methods in influenza type A viruses taxonomy (in Russian). *Problemy Grippa i ORZ* **21**, 14–19.

Popov, V. F., Alekseeva, R. B., Taranova, G. P., and Smirnova, L. B. (1976): Comparative study of biological properties of influenza type B virus strains isolated in the U.S.S.R. and People's Republic of Bulgaria in 1973–1974 (in Russian). *Standarty, Shtammy i Metody Kontrolya bakteriynnykh i virusnykh Preparatov* **2**, 6–11.

Popov, V. F., Taranova, G. P., Smirnova, L. B., Lonskaya, N. I., and Dolmatovskaya, N. V. (1977): Study of the antigenic properties of epidemic influenza virus strains isolated in

1974—1977 (in Russian). *Standarty, Shtammy i Metody Kontrolya bakteriynykh i virusnykh Preparatov* **3**, 21—27.

Reed, L. J., and Muench, H. (1938): A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* **27**, 493—497.

Seto, M., Hariuyma, Ch., Nakamura, T., and Fukuda, J. (1975): Studies on the 1973 type B influenza epidemic in Kawasaki City. *Kitasato Arch. exp. Med.* **48**, 31—41.

Schild, G. C., Pereira, M. S., Chakraverty, P., Coleman, M. T., Dowdle, W. R., and Chang, W. K. (1973): Antigenic variants of influenza B virus. *Brit. Med. J.* **4**, 127—131.

Studier, F. W. (1973): Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J. molec. Biol.* **79**, 237—248.

Tobita, K., and Kilbourne, E. D. (1975): Structural polypeptides of antigenically distinct strains of influenza B virus. *Arch. Virol.* **47**, 367—374.

Voroshilova, M. K., Zhevandrova, V. I., and Balayan, M. S. (1964): *Methods of Laboratory Diagnosis of Enterovirus Infections* (*in Russian*). Meditsina, Moscow.

Weber, K., and Osborn, M. (1969): The reliability of molecular weight determination by dodecylsulfate-polyacrylamide gel electrophoresis. *J. biol. Chem.* **244**, 4406—4412.

Yatel, T. P. (1978): Comparative study of influenza B virus strains isolated in Kiev, 1972—1977 (in Russian). *Mikrobiol. Zh. A.N. U.S.S.R.* **40**, 38—42.

Explanation of Micrographs (Plate XXXIX):

Fig. 1. PAGE of influenza B virus polypeptides (10% gel) run for 4 hr at a constant voltage of 125 V.
Tracks: I — MW markers in kilodaltons. II — B/Lee/40, III — B/Msk/59, IV — B/HK/72, V — B/Len/74, VI — B/Len/75, VII — B/Len/76, VIII — B/Mur/76, IX — B/Sv/76, X — B/Khab/76.
Polypeptides: 1 — NP, 2 — NA, 3 — HA_1, 4 — HA_2, 5 — M.

Fig. 2. PAGE (10% gel) of B/Len/2/75 NA (II) run in parallel with intact virus (I).