Acta virol. **36:** 145-151, 1992

ANTIGENIC AND MOLECULAR ANALYSIS OF INFLUENZA A(H3N2) VIRUS STRAINS ISOLATED IN 1985 IN OPEN AND CLOSED COMMUNITIES OF NORTHERN GERMANY

A. M. HEIDER*, B. HERRMAN**, L. DÖHNER**, [1]A. I. KLIMOV*

*Research Institute for Viral Preparations, Academy of Medical Sciences, 109088 Moscow, Russia; and **Institute for Medical Microbiology, Ernst-Moritz-Arndt University, Greifswald, Germany

Received November 29, 1990

Summary. - Antigenic and molecular analyses of influenza A(H3N2) virus strains isolated in 1985 during outbreaks in open and closed communities of North Germany were carried out. The data obtained have shown that 11 strains isolated in a closed orphanage were antigenically similar to each other. The electrophoretic mobilities of either HA, NP, M1 and NS1 polypeptides or of double stranded RNA segments were indistinguishable. Analysis of viruses isolated at the same time from open communities has revealed that they contained at least three groups of strains differing in homology of 3-5 RNA segments. These data support the idea that an outbreak of influenza in a community is caused by single virus strain, from which their slightly different variants of the virus arise during circulation among sensitive persons.

Key words: influenza virus variation; community; outbreak of infection

Introduction

Recent investigations of influenza A and B virus strains isolated during epidemic seasons in open communities have revealed significant heterogeneity not only in antigenic specificity of surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), but also in the genes coding for nonglycosylated proteins (Ghendon *et al.*, 1981; Klimov and Ghendon, 1985; Oxford *et al.*, 1984; Pereira *et al.*, 1985).

Such heterogeneity may be caused either by appearance of new virus variants in the course of an epidemic (Young *et al.*, 1979) or by activation of one from several pre-existing virus variants (Bao-Lang *et al.*, 1983). The investigation of strains circulating during an epidemic in a closed community might

[1]Whom requests for reprints should be addressed.

clarify this question. Antigenic and genomic analyses of influenza A virus strains isolated during an outbreak in a closed community of carmelite nuns in Rome revealed significant similarity of these strains (Donatelly *et al.*, 1990). Analysis of influenza B virus isolates from local epidemic in boarding schools also has shown considerable genome homogeneity of variants circulating within the boundaries of each college (Oxford *et al.*, 1983; Tikhonova-Heider *et al.*, 1986). These data testify that infection can be initiated by a single influenza virus strain. Moreover, in one of these schools there were successful attempts to identify, at late stages of the epidemic, variants with slightly differing genomes from the majority (Tikhonova-Heider *et al.*, 1986). The last results suggest that heterogeneity within a viral population is caused by selection of new influenza virus variants during an epidemic. To show that methods used by us earlier are sensitive enough to differentiate between heterogenic and homogenic virus populations, in this study, we carried out a comparative antigenic, polypeptide and genome analysis of influenza A(H3N2) virus strains isolated in 1985 in open and closed communities of Greifswald (North Germany) and in two small towns in the neighbourhood of Greifswald.

Materials and Methods

Viruses. Influenza A(H3N2) virus strains were used in this study. Data and places of their isolation as well as the age of patients from whom these strains have been isolated are presented in Table 1. Nasopharyngeal washes were taken from patients in polyclinics of towns Greifswald and Bergen (island Rügen, 63 km from Greifswald) as well as from infants residing in a closed community (an orphanage) in the town Garz (island Rügen, 17 km from Bergen and 55 km from Greifswald). Isolation of strains was performed in the Institute for Medical Microbiology (Greifswald). The viruses were grown in allantoic sacks of 11-days old embryonated eggs at 33 °C for 48 hr. The strains underwent not more than 4 passages in eggs after isolation. A/Bangkok/1/79 (H3N2) was used as a reference virus.

Haemagglutination inhibition (HI) tests were carried out in accordance with the standard method in the presence of rabbit antiserum against influenza A(H3N2) viruses: A/Bangkok/1/79, A/Berlin/3/82, A/Dresden/1/83 and A/Greifswald/19/85.

Analysis of ^{35}S-labelled virus-specific polypeptides was made in 16.5 % PAG according to the method described by Seidel *et al.* (1985). Autoradiography was carried out as described by Russel and Skehel (1972).

Analysis of double stranded RNAs was performed as described by Hay *et al.* (1977) and Ghendon *et al.* (1979). Virion RNA (vRNA) was isolated from purified viral particles and hybridized with complementary RNA (cRNA) isolated from CEF cells infected with corresponding virus strain (100 ID_{50}/cell) and incubated in the presence of cycloheximide (100 mg/ml) and ^{3}H-uridine (3.7 MBk/ml). Hybrid doublestranded (ds) RNAs were treated with nuclease S1 (1000 U/ml, 37 °C, 4 hr) and electrophoresed in 7.5 % PAG containing 5 M urea. The treatment of gels for radiography was done according to the method of Bonner and Laskey (1970).

Results

According to HI test, the data of all strains under study isolated in 1985 in open or closed communities, differed considerably from the A/Bangkok/1/79,

Table 1. Influenza A (H3N2) virus atrains isolated in 1985 in closed and open communities in the Greifswald area

Strain	Date of isolation	Patients age (years)	Place of isolation
A/Greifswald/1/85	23.01.85	4	Garz, orphanage
A/Greifswald/2/85	23.01.85	3	"
A/Greifswald/3/85	23.01.85	5	"
A/Greifswald/4/85	23.01.85	4	"
A/Greifswald/6/85	23.01.85	4	"
A/Greifswald/7/85	23.01.85	4	"
A/Greifswald/8/85	23.01.85	6	"
A/Greifswald/9/85	23.01.85	3	"
A/Greifswald/10/85	23.01.85	4	"
A/Greifswald/11/85	23.01.85	4	"
A/Greifswald/12/85	23.01.85	5	"
A/Greifswald/18/85	9.01.85	2	Greifswald, outpatient
A/Greifswald/14/85	10.01.85	4	"
A/Greifswald/22/85	1.02.85	3	"
A/Greifswald/35/85	1.02.85	3	"
A/Greifswald/39/85	12.02.85	6	"
A/Greifswald/40/85	12.02.85	1	"
A/Greifswald/57/85	6.03.85	3	"
A/Greifswald/31/85	19.02.85	18	Bergen, outpatient
A/Greifswald/36/85	19.02.85	25	"

Nasopharengal washes were taken in outpatient departments (open communities) of Greifswald and Bergen (island Rügen) and an orphanage (closed community) in Garz (island Rügen).

A/Berlin/3/82 and A/Dresden/1/83 viruses isolated in previous years. At the same time there were only minor antigenic differences between the year 1985 isolates (Table 2).

Polypeptide analysis of the isolates in 16.5 % PAG has revealed their similarity in the mobility of HA, NP and M1 proteins. Nevertheless, all strains investigated could be divided into two groups based on the NS1 polypeptide mobility: the first group included all strains isolated in an orphanage and 5 from 9 strains (A/Greifswald/14/85, A/Greifswald/18/85, A/Greifswald/36/85, A/Greifswald/39/85, A/Greifswald/57/85) isolated in an open community. The second group strains showing a different NS1 mobility were all isolated in outpatient departments (Fig. 1).

Analysis in 7.5 % PAG of dsRNAs obtained after the hybridization of 3H-cRNAs of „orphanage strains" with vRNA of the A/Greifswald/12/85 virus revealed the identity of these strains to each other and homology of all their genes (Fig. 2 presents the data concerning strains A/Greifswald/2/85, A/

Table 2. Antigenic specificity of influenza A (H3N2) strains isolated in 1985 in the Greifswald area

Strain	HI titers with sera against			
	A/Bangkok/79	A/Berlin/82	A/Dresden/83	A/Greifswald/9/85
A/Bangkok/79	**2560**	1280	5120	1280
A/Berlin/82	960	**1920**	1280	1280
A/Dresden/82	1280	480	**2560**	960
A/Greifswald/2/85	320	160	640	1280
A/Greifswald/3/85	320	160	640	1280
A/Greifswald/12/85	320	160	480	960
A/Greifswald/14/85	480	240	480	960
A/Greifswald/18/85	640	320	640	1280
A/Greifswald/19/85	320	160	640	**1280**
A/Greifswald/22/85	640	320	640	1280
A/Greifswald/31/85	320	320	640	1280
A/Greifswald/35/85	320	160	320	640
A/Greifswald/36/85	320	320	640	1280
A/Greifswald/39/85	640	320	640	1280
A/Greifswald/40/85	320	640	640	1280
A/Greifswald/57/85	320	160	640	1280

Greifswald/3/85, A/Greifswald/9/85 and A/Greifswald/12/85, but data were obtained for all viruses isolated in this orphanage). It should be noted that these strains differed from the reference A/Bangkok/1/79 virus in the homology of all their 8 RNA segments (Fig. 2).

At the same time, the dsRNA analysis has shown that all strains isolated in open communities can be classified into three groups. The first group joins strains A/Greifswald/14/85, A/Greifswald/18/85, A/Greifswald/36/85 and A/Greifswald/57/85; the second one includes isolates A/Greifswald/22/85, A/Greifswald/32/85 and A/Greifswald/35/85; and the third group consists of strains A/Greifswald/39/85 and A/Greifswald/40/85. Fig. 2 demonstrates results of the genome analysis for some strains representing these three groups. The A/Greifswald/18/85 isolate (the first group) differs from the A/Greifswald/35/85 strain (the second group) by the RNA segments 1, 2, 3, 7 and 8, but from the A/Greifswald/40/85 isolate (the third group) by RNA segments 1, 3, 7 and 8. Isolates of the second (A/Greifswald/5/85) and the third (A/Greifswald/40/85) groups differ each from other only by RNA segments 3 and probably 8 (Fig. 2).

Thus, among viruses isolated during the 1985 influenza epidemic in open communities of Greifswald and its vicinities, there have been at least two

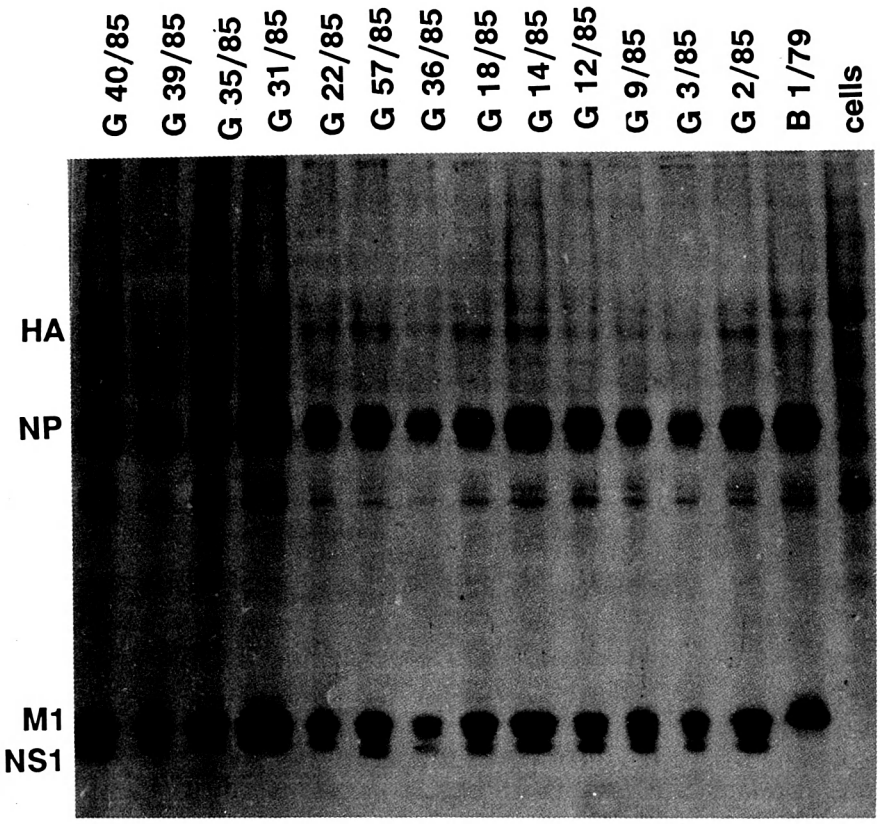

Fig. 1
Polypeptide analysis in 16.5 % PAG of influenza A (H3N2) viruses isolated during an 1986 outbreak in the Greifswald area
Designation of strains: B 1/79 - A/Bangkok/1/79; G 2/85 - A/Greifswald/2/85; G 3/85 - A/Greifswald/3/85; G 9/85 - A/Greifswald/9/85; etc.

groups of isolates differing in electrophoretic mobility of the NS1 protein and three groups of isolates differing in the homology of 3-5 RNA segments.

Discussion

The results obtained allow us to conclude that the degree of influenza virus heterogeneity might depend on the epidemiological circumstances of the group in which the virus is circulating. The virus population in a closed community is homogeneous. All strains isolated in the orphanage were iden-

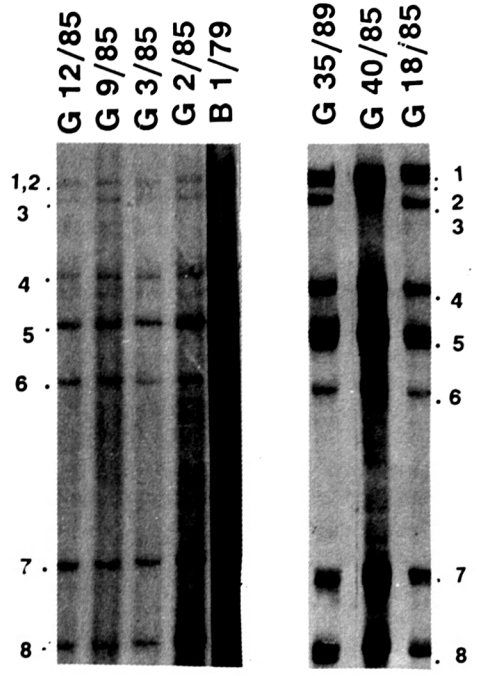

Fig. 2
Analysis in 7.5% PAG of dsRNAs obtained after the hybridization of ^{3}H-cRNAs of influenza A/H3N2) viruses isolated during an 1985 outbreak in the Greifswald area with the vRNA of the A/Greifswald/12/85 strain (in the left) or with vRNA of the A/Greisfwald/18/85 strain (in the right)
Designation of strains (^{3}H-cRNAs): B 1/79 - A/Bangkok/1/79; G 2/85 - A/Greifswald/2/85; G 3/85 - A/Greifswald/3/85; G 9/85 - A/Greifswald/9/85; etc.

tical each to other in the homology of all genes and in the electrophoretic mobility of virus polypeptides. However, among viruses which had been isolated during the same epidemic in an open community at least three groups of isolates differing in electrophoretic mobility of NS1 protein or virus-specific ds RNAs were detected.

It is interesting that the strains isolated during the 1985 winter in the Greifswald area in open and closed communities did not differ antigenically in HI test with polyclonal sera against A(H3N2) viruses. They also did not differ in the mobility of HA in PAG and in the homology of their HA-genes. At the same time, the strains isolated in an open community differed by genes coding for „internal" proteins (PB2, PB1, PA, M, NS). These data are indicative of the variability of genes coding for non-glycosylated influenza A virus proteins, that not only might be independent on the evolution of HA- and NA-genes (Klimov and Ghendon, 1985) but sometimes probably might not be accompanied by variability of the latter.

All strains have been isolated, passaged and studied under very similar conditions. This allows the conclusion that heterogeneity (sometimes significant one) of virus populations circulating in open communities is not a laboratory artifact which could be caused, for example, by adaptation of human influenza virus strains to a heterologous culture when simultaneuosly isolated

and cultivated in a similar way. Strains obtained in a closed community manifest high genetic similarity to each other (Tikhonova-Heider *et al.,* 1986).

The data presented here support the idea (Young *et al.,* 1979; Tikhonova-Heider *et al.,* 1986) that an influenza outbreak might be caused by a single virus variant, from which different variants of the virus arise during its circulation among susceptible persons.

Acknowledgements. We thank Dr. Paul Rota for useful discussion and help in the manuscript preparation.

References

Bao-Lan, L., Webster, R. G., Brown, L. E., and Nerome, K. (1983): Heterogeneity of influenza B viruses. *Bull. World Hlth. Org.* **61**, 681-684.

Bonner, W. M., and Laskey, P. H. (1970): A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Europ. J. Biochem.* **46**, 83-86.

Donatelli, I., Tikhonova, A. M., Klimov, A. I., Ghendon, Y. Z., and Oxford, J. S. (1990): Genome and antigenic analysis of influenza A(H3N2) viruses isolated from an epidemic in a closed community of Carmelite nuns. *J. med. Virol.* **31**, 112-119.

Ghendon, Y. Z., Klimov, A. I., Gorodkova, N. V., and Döhner, L. (1981): Genome analysis of influenza A virus strains isolated during an epidemic of 1979-1980. *J. gen. Virol.* **56**, 303-313.

Hay, A. J., Lomniczi, B., Bellamy, A. R., and Skehel, J. J. (1977): Transcription of influenza virus genome. *Virology* **83**, 337-345.

Klimov, A. I., and Ghendon, Y. Z. (1985): Analysis of pecularities of influenza A virus strains (H3N2) isolated during an epidemic season of 1982-1983. *Arch. Virol.* **86**, 253-262.

Oxford, J. S., Abbo, H., Corcoran, T., Webster, R. G., Smith, J., Grilli, E. A., and Shild, G. C. (1983): Antigenic and biochemical analysis of field isolates of influenza B virus: evidence for intra- and interepidemic variation. *J. gen. Virol.* **64**, 2367-2377.

Oxford, J. S., Klimov, A. I., Corcoran, T., Ghendon, X. Z., and Schild, G. C. (1984): Biochemical and serological studies of influenza B viruses: comparison of historical and recent isolates. *Virus Res.* **1**, 241-258.

Pereira, M. S., Chakraverty, P., Cunningham, P., and Webster, R. G. (1985): The use of monoclonal antibodies for antigenic analysis of influenza A viruses. *Bull. World Hlth. Org.* **63**, 265-271.

Russel, W. C., and Skehel, J. J. (1972): The polypeptides of adenovirus infected cells. *J. gen. Virol.* **15**, 45-57.

Seidel, W., Herrman, B., Langhammer, J., Geisler, B., Döhner, L., and Ghendon, Y. (1985): Variation of influenza A(H3N2) viruses isolated in the GDR during 1979-1980 epidemics. *Acta virol.* **30**, 177-186.

Tikhonova-Heider, A. M., Klimov, A. I., Smith, A. J., Grilli, E., Oxford, J. S., and Ghendon, Y. Z. (1986): Genome analysis of influenza B virus strains isolated in boarding schools. *Arch. Virol.* **89**, 255-262.

Young, J. F., Desselberger, U., and Palese, P. (1979): Evolution of human influenza A viruses in nature: sequential mutations in the genome of new H1N1 isolates. *Cell* **18**, 73-76.