Infection, Genetics and Evolution 12 (2012) 1694–1698

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Molecular genetic analysis of influenza A/H3N2 virus strains isolated in Western Siberia in the 2010–2011 epidemic season

I. Sobolev [a], O. Kurskaya [a], I. Susloparov [a], T. Ilyicheva [a,b,*], A. Shestopalov [a]

[a] State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk 630559, Russia
[b] Novosibirsk State University, Pirogov St. 2, Novosibirsk 630090, Russia

ARTICLE INFO

Article history:
Received 26 February 2012
Received in revised form 15 July 2012
Accepted 23 July 2012
Available online 16 August 2012

Keywords:
A/H3N2 influenza virus
Molecular genetic analysis
Western Siberia

ABSTRACT

Molecular genetic and antigenic features of influenza A/H3N2 virus strains isolated in Western Siberia in 2011 are similar to those of the vaccine strain A/Perth/16/2009 despite a number of unique amino-acid changes. The strains lack specific amino-acid changes in NA protein providing decrease of sensibility to NA inhibitors activity that used in medical practice. Based on phylogenic analysis of HA protein amino-acid sequences examined strains are similar to influenza A/H3N2 virus strains circulating at the moment in Eurasia.

1. Introduction

Influenza is currently one of the major public health concerns. The epidemic nature of influenza morbidity results in a variety of problems both in social and economic fields: mortality in risk group patients (age younger than 2 and greater than 65 years, people with disbolism and compromised immunity), the dramatic increase in workload for public health personnel, and labor resources decrease.

Influenza viruses that are a member of the *Orthomyxoviridae* family are divided into A, B, and C types. Influenza A viruses have wide range of hosts and are able to infect birds, animals, and human and are divided into subtypes on the basis of antigenic characteristic of surface glycoproteins – hemagglutinin (HA) and neuraminidase (NA) Webster et al. (1992). At the moment primarily influenza virus subtypes A/H3N2 and A/H1N1 as well as influenza type B viruses circulate in human population (Lin et al., 2004).

We have carried out influenza molecular surveillance in Western Siberia since 2008. In 2008–2009, we isolated and described 17 influenza virus strains: 9 A/H1N1 influenza virus strains; 5 A/H3N2 influenza viruses, and 3 influenza B viruses (Il'icheva et al., 2010). In 2009–2010, when influenza A/H1N1pdm09 virus pandemic began, we isolated only pandemic influenza viruses from patients. In total 56 strains were isolated and described (Ilyicheva et al., 2011). In the 2010–2011 season substantially A(H1N1)dpm09 (86.5%) viruses were isolated; there were only 5.8% of influenza A/H3N2 virus strains, and 9% of influenza B viruses.

The aim of this work was to study influenza A/H3N2 virus strains having circulated in Western Siberia in 2008–2009 and 2010–2011 seasons.

2. Materials and methods

Influenza virus strains were isolated from infected people in the 2010–2011 epidemic season (written consent of all patients had been obtained). During epidemic season under study 256 clinical samples were obtained in Western Siberia. Fifty two influenza virus strains were isolated from those samples: 4 influenza type B virus strains, 45 influenza A/H1N1 virus strains, and 3 influenza A/H3N2 virus strains. In this paper we demonstrate results of molecular genetic analysis of isolated influenza A/H3N2 virus strains. Viruses were passaged on Madin–Darby canine kidney (MDCK) cells as described previously (Ilyicheva et al., 2011). Antigenic specificity of isolated strains was determined in the hemagglutination inhibition (HI) test with reference sera and guinea pig red blood cells using standard practice (Schwahn and Downard, 2009).

Influenza virus RNA isolation was carried out with the kit PROMEGA SV Total RNA Isolation System (Promega Corporation, Madison, WI, USA) in compliance with the manufacturer's recommendations.

To type and subtype studied influenza virus strains we used a set of reagents for the detection of influenza A and influenza B virus RNA in clinical materials using PCR with hybridization-fluorescence detection AmpliSens® Influenza virus A/B-FL and the

* Corresponding author at: Novosibirsk State University, Pirogov St. 2, Novosibirsk 630090, Russia. Tel./fax: +7 383 3367540.
E-mail address: ilyichev@mail.ru (T. Ilyicheva).

1567-1348/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2012.07.014

kit for typing (subtype identification of H1N1 and H3N2) of influenza A viruses AmpliSens® Influenza virus A-type-FL made by Central research institute of epidemiology of the Ministry of health of Russia (Moscow, Russia).

To produce cDNA from influenza virus RNA matrix reverse transcription was carried out with the kit Fermentas RevertAid (Fermentas International Inc., Vilnius, Lithuania).

To amplify certain gene segments encoding influenza virus surface glycoproteins PCR with gene-specific primers was used (Ghedin et al., 2005). We used the following primer sequences:

NA_F_1M13	TGTAAAACGACGGCCAGTAGCRAAAGCAGG
NA_R_560M13	CAGGAAACAGCTATGACCTCGTGACAACTTGA GCTGGAC
NA_F_415M13	TGTAAAACGACGGCCAGTTATCAATTTGCMCT TGGRCAGG
NA_R_984M13	CAGGAAACAGCTATGACCAAGYCCTGAGCACA CATARC
NA_F_880BM13	TGTAAAACGACGGCCAGTTCAGATGTRTHTGC MGAGAC
NA_R_1465BM13	CAGGAAACAGCTATGACCAGTAGAAACAAGGA GTTTTT
HA_F_1BM13	TGTAAAACGACGGCCAGTAGCARAAGCAGGG GA
HA_R_589M13	CAGGAAACAGCTATGACCTTGTTTGGCATRGT CACGTTC
HA_F_453BM13	TGTAAAACGACGGCCAGTTTCRAYTGGRCTGG RGTCRC
HA_R_975M13	CAGGAAACAGCTATGACCTTTTGAAADGGYTT GTCATTGG
HA_F_872M13	TGTAAAACGACGGCCAGTAAGCTCRATAATGA GRTCAGAT
HA_R_1425M13	CAGGAAACAGCTATGACCAGTCAGTYAGATSA ATTGTATGTTG
HA_F_1300M13	TGTAAAACGACGGCCAGTTTCAGGACCTCGAG AAATAYG
HA_R_1778BM13	CAGGAAACAGCTATGACCAGTAGAAACAAGG GTGTTTT

Amplification was carried out with DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad, USA).

In order to extract gene segments obtained by PCR which were coding NA and HA of studied influenza A/H3N2 virus strains a set of reagents QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden) was used.

Sequence analysis was conducted with BigDye terminator cycle sequencing ready reaction kit (Applied BioSystems, USA). Amplification passed in accordance with the manufacturer's recommendations. To purify the product the BigDye XTerminator purification kit (Applied BioSystems, USA) was used. Automatic sequenator 310 Genetic Analyzer (Applied BioSystems, USA) was used for the analysis of products.

Analysis of nucleotide sequences was carried out with the program package SeqMan (Lasergene) and specific Influenza Virus Resource (Bao et al., 2008) and BLAST (National Center for Biotechnology Information, U.S. National Library of Medicine). To carry out multiple sequence alignment and nucleotide sequence translation as well as to analyze obtained amino-acid sequences we used BioEdit sequence alignment editor.

Phylogenetic trees based on adjusted nucleotide sequences were built with the program MEGA5 using the neighbor-joining method (model: Maximum composite likelihood) (Saitou and Nei, 1987). To value certainty bootstrap test (1000 replications) was used.

3. Results

Novosibirsk region occupies an area of about 180,000 sq. km. The territory of the region extends for 642 km from west to east, and 444 from north to south. The population numbers 2.7 million that is more than 20% of Western Siberia population. Influenza virus strains under our study were isolated in different settlements of Novosibirsk region separated by hundreds of kilometers from each other. Nasopharyngeal swabs were collected in four Novosibirsk region hospitals: Koltsovo, Dovolnoe, Zdvinsk regional hospitals, and central city hospital in Novosibirsk. In total 256 samples were collected from patients with influenza-like illness.

We studied three influenza A/H3N2 virus strains isolated in Western Siberia in the 2010–2011 epidemic season: A/Novosibirsk/76/2011, A/Novosibirsk/1832/2011, A/Novosibirsk/1927/2011. Strain A/Novosibirsk/76/2011 (H3N2) was isolated 08.02.2011 from 34-year-old woman; strain A/Novosibirsk/1832/2011 (H3N2) was isolated 15.04.2011 from 8-year-old boy; strain A/Novosibirsk/1927/2011 (H3N2) was isolated 21.04.2011 from 23-year-old man. All patients developed influenza-like illness with benign outcome. The strains were typed and subtyped in the HI test. PCR with real-time results detection was used to verify HI test result; it was shown that the studied strains belong to influenza A/H3N2 virus.

We detected full nucleotide sequence of NA and HA genes encoding respective surface glycoproteins of influenza virus (GenBank accession numbers: JN940428–JN940432) and performed comparative analysis of influenza A/H3N2 virus strains isolated in the 2008–2009 (CY053652, CY053653, CY053660, CY053661, CY053662, CY053663, CY053666, CY053667, CY053672, CY053673) (Il'icheva et al., 2010) and 2010–2011 epidemic seasons.

Table 1
Amino-acid substitutions in NA of influenza A/H3N2 virus strains isolated in Novosibirsk region in the epidemic season of 2008–2009 (A/Novosibirsk/31/2009, A/Novosibirsk/319/2009, A/Novosibirsk/628/2009, A/Novosibirsk/707/2009, A/Novosibirsk/1211/2009), 2010–2011 (A/Novosibirsk/76/2011, A/Novosibirsk/1832/2011, A/Novosibirsk/1927/2011) compared to the vaccine strains A/Brisbane/10/2007 and A/Perth/16/2009. Dark gray, amino-acid changes that distinguish strains isolated in Novosibirsk region in the epidemic season of 2010–2011 from vaccine strain A/Perth/16/2009. Bright gray, changes distinguishing strains of 2008 – 2009 from vaccine strain A/Brisbane/10/2007.

Virus	30	127	147	151	215	221	307	312	315	325	338	339	342	386	402	430	463	464
A/Brisbane/10/2007	I	D	D	D	I	E	I	T	S	T	L	D	N	P	N	R	D	I
A/Novosibirsk/31/2009	I	D	N	D	V	E	I	I	S	T	L	D	N	H	N	R	D	L
A/Novosibirsk/319/2009	I	D	N	G	V	E	I	I	S	T	L	D	N	P	N	R	D	I
A/Novosibirsk/628/2009	I	D	N	G	V	D	I	I	S	T	L	N	N	P	N	R	N	I
A/Novosibirsk/707/2009	I	D	N	A	V	D	I	I	S	T	L	N	N	P	N	R	N	I
A/Novosibirsk/1211/2009	V	D	N	D	V	D	I	I	R	P	L	D	N	P	N	R	N	I
A/Perth/16/2009	I	D	N	D	V	E	I	I	S	T	L	D	N	P	N	R	D	I
A/Novosibirsk/76К/2011	I	N	N	D	V	E	M	I	S	T	F	D	D	P	D	S	D	I
A/Novosibirsk/1832/2011	I	N	N	D	V	E	M	I	S	T	F	D	D	P	D	S	D	I
A/Novosibirsk/1927/2011	I	N	N	D	V	E	M	I	S	T	F	D	D	P	D	S	D	I