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Human influenza A viruses isolated in South America: Genetic relations, adamantane resistance and vaccine strain match☆

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ABSTRACT

In order to gain insight into the genetic relations among H3N2 Influenza A virus (IAV) circulating in the South American region from 1999 to 2007, to investigate the presence of adamantane-resistant strains in this region, and to establish the genetic relations among that strains and vaccine strains recommended for the Southern hemisphere, 11 haemagglutinin (HA) H3 IAV sequences obtained from Uruguayan patients were aligned with corresponding sequences from 68 H3 IAV strains isolated in South America and 9 H3 IAV vaccine strains. Maximum likelihood phylogenetic tree analysis was performed using the GTR evolutionary model. The results of these studies indicate that multiple clades co-circulate during most influenza seasons in South America. Strikingly, one strain isolated in Uruguay in 2005 and all strains isolated in that country during the 2007 season bear an HA adamantane-resistant polymorphism. No other strain isolated in South America previous to the 2005 season bears that HA characteristic amino acid change. Only vaccine strains recommended for the 2007 season were assigned to the same cluster with all available IAV isolated in South America for that season. Evolution of IAV in this region appears to be shaped by re-introduction of new strains.

1. Introduction

Influenza A virus (IAV) is a member of the family *Orthomyxoviridae* and contains eight segments of a single-stranded RNA genome with negative polarity (Neumann et al., 2004). IAV causes 300,000–500,000 deaths worldwide each year, and in pandemic years, this number can increase to 1 million (in 1957–1958) or as high as 50 million, as was seen in 1918–1919 (Nguyen-Van-Tam and Hampson, 2003; WHO, 2007). IAV evades host immunity by accumulation of point mutations (drift) in the major surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), or as a result of genetic reassortment of segments from different IAV strains co-infecting the same cell (shift) (Nicholson et al., 2003). New IAV pandemics may emerge through reassortation with strains from the avian reservoir or by direct introduction of an avian strain into the human population (Taubenberger and Morens, 2006).

At present time only 2 of 16 possible HA subtypes (H1 and H3) and 2 of 9 possible NA (N1 and N2) described to date are circulating in the human population. H3N2 and H1N1 IAV have co-circulated in humans since the re-emergence of H1N1 in 1977. IAV H3N2 viruses have been the predominant strains during the last 20 years, with the exception of the 1988–1989 and 2000–2001 seasons where H1N1 infections dominated (Lin et al., 2004). Based on antigenic analyses of recently isolated influenza viruses, epidemiologic data, post-vaccination serologic studies in humans, and the availability of candidate vaccine strains, the World Health Organization (WHO) recommend the influenza virus strains to be included in the trivalent influenza vaccine, composed of one H1N1 and one H3N2 IAV strains plus one Influenza B virus strain, for each Northern hemisphere winter season (available at: http://www.who.int/csr/disease/influenza/vaccinerecommendations1/en/index.html).

Since October 1998, a second meeting of WHO is held to evaluate the vaccine formula in order to recommend the influenza virus strains to be included in the vaccine for the Southern hemisphere (Pontoriero et al., 2003; available at: http://

☆ *Note*: Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the accession numbers AM991338 through AM991342.

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www.who.int/csr/disease/influenza/vaccinerecommendations1/en/index.html).

Adamantanes, like amantadine or rimantadine, have been used as anti-viral agents against IAV in developed countries (Hata et al., 2007). Adamantanes block the ion channel of IAV M2 protein and thus inhibit the pH change necessary for the uncoating process (Wang et al., 1993). A dramatic rise in the frequency of resistance to adamantane drugs by H3N2 IAV has occurred in recent years in different countries (Simonsen et al., 2007). In the influenza 2005–2006 season, an important increase of resistant viruses in communities was observed in Japan (Saito et al., 2007), Southeast Asia and Australia (Barr et al., 2007), and North America (Bright et al., 2006). Furthermore, the resistant viruses were already detected in September 2005, just before the influenza season in Nagasaki, Japan, and even in previous 2004–2005 influenza season in Aichi prefecture in the same country (Hata et al., 2007). Phylogenetic analysis revealed that the resistant viruses belong to a single genetic lineage, sharing a mutation at amino acid position 31 of the M2 protein (S31N) (Simonsen et al., 2007; Hata et al., 2007). These analyses have also shown that these viruses also share common characteristic amino acid changes at positions 193 (S193F) and 225 (D225N) of the HA protein (Hata et al., 2007). Little is know about the genetic relations among H3N2 IAV strains circulating in the South American region, the presence and frequency of adamantane-resistant strains in this region, as well as the genetic relations among South American IAV strains and vaccine strains recommended for the Southern hemisphere.

2. Materials and methods

2.1. Human samples

Nasal swabs from 11 Uruguayan patients with clinical symptoms of influenza were available at the National Influenza Reference Centre, Montevideo, Uruguay.

2.2. Virus type

In order to address virus type, nasal swabs were first cultured in MDCK cells. Viral antigens were detected by an immunofluorescent assay with type-specific monoclonal antibodies (Chemicon International, Inc., CA, USA) and with the Directigen Flu A+B (Beckton Dickinson Europe, Maylan, France). Virus isolates were typed by haemagglutination inhibition assay (HAI) with the WHO Influenza reagent kit, provided by the Center for Disease Control and Prevention (CDC), Atlanta, GA, USA All 11 IAV strains isolated from the Uruguayan patients were assigned to subtype H3.

2.3. RNA extraction and RT-PCR amplification

Total RNA was extracted from infected cells using Trizol (Gibco, BRL) according to manufacturer's instructions. Extracted RNA was eluted with 15 μl of UltraPure DNAse/RNase-free distilled water (Gibco, BRL, Life Technologies) and cDNA synthesis and PCR amplification of IAV HA gene was carried out as previously described (Ellis et al., 1997). Amplicons were purified by using a QIAquick Gel Extraction Kit (QIAGEN) according to the manufacturer's instructions prior to sequencing.

2.4. Sequencing reactions

Purified PCR products were sequenced directly. The primers used for amplification were also used for sequencing the PCR fragments. The sequencing reaction was carried out using a BigDye DNA sequencing kit on a 373 DNA Sequencer Apparatus, both from PerkinElmer.

2.5. Nucleotide sequence accession numbers

Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the accession numbers AM991338 through AM991342.

For sequence names and accession numbers see Table 1.

Table 1
Origins of the H3 IAV strains isolated in South America.

Name	Accession number	Country of isolation
A/Rio de Janeiro/57/1999	AY968022	Brazil
A/Espirito Santo/33/1999	AY968021	Brazil
A/Rio Grande do Sul/25/1999	AY968020	Brazil
A/Rio Grande do Sul/21/1999	AY968019	Brazil
A/Espirito Santo/14/1999	AY968018	Brazil
A/Espirito Santo/3/1999	AY968017	Brazil
A/Buenos Aires/M6/1999	AF534032	Argentina
A/Chaco/140/1999	AF534035	Argentina
A/Buenos Aires/M14/1999	AF534034	Argentina
A/Mar del Plata/267/1999	AF534040	Argentina
A/Brazil/003/2000	DQ336007	Brazil
A/Brazil/024/2000	DQ336007	Brazil
A/Brazil/011/2000	DQ336016	Brazil
A/Brazil/013/2000	DQ336017	Brazil
A/Brazil/006/2000	DQ336015	Brazil
A/Brazil/010/2000	DQ336013	Brazil
A/Brazil/008/2000	DQ336011	Brazil
A/Rio de Janeiro/28/2000	AY968023	Brazil
A/Espirito Santo/128/2000	AY968024	Brazil
A/Rio de Janeiro/172/2000	AY968025	Brazil
A/Rio de Janeiro/580/2001	AY968036	Brazil
A/Rio Grade do Sul/523/2001	AY968033	Brazil
A/Chile/6416/2001	DQ865972	Chile
A/Espirito Santo/452/2001	AY968027	Brazil
A/Rio de Janeiro/565/2001	AY968035	Brazil
A/Rio de Janeiro/471/2001	AY968031	Brazil
A/Rio de Janeiro/470/2001	AY968030	Brazil
A/Rio de Janeiro/465/2001	AY968029	Brazil
A/Rio de Janeiro/310/2001	AY968026	Brazil
A/Espirito Santo/454/2001	AY968028	Brazil
A/Brazil/125/2001	DQ330006	Brazil
A/Rio de Janeiro/533/2001	AY068040	Brazil
A/Rio de Janeiro/478/2001	AY968032	Brazil
A/Rio Grande do Sul/523/2001	AY968033	Brazil
A/Brazil/722/2001	AF534056	Brazil
A/Neuquen/1016002/2001	AF534059	Argentina
A/Cordoba/1007333/2001	AF534058	Argentina
A/Neuquen/1038228/2001	AF534060	Argentina
A/Neuquen/2260/2001	AF534057	Argentina
A/Chile/6416/2001	DQ865972	Chile
A/Chaco/R538/2001	AF534055	Argentina
A/Santa Catarina/339/2002	AY968040	Brazil
A/Espirito Santo/88/2002	AY968041	Brazil
A/Santa Catarina/311/2002	AY968038	Brazil
A/Rio Grande do Sul/205/2002	AY968033	Brazil
A/Santa Catarina/327/2002	AY968039	Brazil
A/Rio de Janeiro/98/2003	AY972834	Brazil
A/Rio de Janeiro/99/2003	AY972832	Brazil
A/Rio de Janeiro/107/2003	AY972833	Brazil
A/Rio de Janeiro/346/2003	AY972851	Brazil
A/Uruguay/11/2003	AM991343	Uruguay
A/Santa Catarina/379/2004	AY972848	Brazil
A/Minas Gerais/156/2004	AY972827	Brazil
A/Minas Gerais/154/2004	AY972829	Brazil
A/Rio de Janeiro/17/2004	AY972847	Brazil
A/Rio Grande do Sul/406/2004	AY972846	Brazil
A/Santa Catarina/380/2004	AY972845	Brazil
A/Parana/306/2004	AY972837	Brazil
A/Parana/308/2004	AY972838	Brazil
A/Parana/312/2004	AY972849	Brazil
A/Rio Grande do Sul/212/2004	AY972835	Brazil
A/Parana/298/2004	AY972830	Brazil
A/Rio Grande do Sul/417/2004	AY972844	Brazil
A/Rio Grande do Sul/411/2004	AY972841	Brazil
A/Rio de Janeiro/26/2004	AY972840	Brazil