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Effect of seasonal vaccination on the selection of influenza A/H3N2 epidemic variants

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ABSTRACT

The effect of vaccination on the dynamics of influenza virus variants remains largely unknown in humans, unlike in poultry. In this study, we compared influenza hemagglutinin (HA) gene sequences isolated from vaccinated and unvaccinated populations with the yearly vaccine strains. In total, 181 influenza A/H3N2 virus samples isolated from 82 vaccinated and 99 unvaccinated patients (2011–15, four Japanese influenza seasons) were genetically analyzed using a next-generation sequencer. Amino acid (AA) differences from corresponding vaccine strains were found in 74 of 329 HA1 sites. There was a maximum of four AA differences within the epitopes in the former three seasons (2011–14) and fifteen in the latter season (2014–15). Deviation to a greater number of AA differences was found more significantly in the isolates from vaccinated patients as compared to unvaccinated patients ($P = 0.0005$ in 2011–14; $P = 0.0096$ in 2014–15). AA difference rates within epitopes were also significantly higher in the isolates from vaccinated patients than from unvaccinated patients (2.64% vs. 2.14% for 2011–14, $P = 0.033$; 7.78% vs. 6.59% for 2014–15, $P = 0.058$). The AA differences at seven sites (48I-278K, 128A-142G, 145S, 158K, and 193S) became dominant in the following seasons. In all of these sites, the dominance was retained during the mismatch of isolates with the vaccine strains and was lost after vaccine match. Our data suggest that in humans, immune pressure induced by vaccination works to select influenza variants genetically distant from vaccine strains.

1. Introduction

Influenza A/H3N2 virus is a major viral pathogen that causes great morbidity and mortality in humans, imposing a considerable socioeconomic burden. Influenza viruses are highly mutable because of the lack of RNA proofreading and can escape host immunity by accumulating mutations in the hemagglutinin (HA) gene, which encodes the main target antigen recognized by the infected host [1]. This mutation process is called antigenic drift. Consequently, influenza vaccine strains are updated through analysis of antigenic drift [2]; however, protection by influenza vaccines, estimated by their effectiveness, has been suboptimal, particularly in recent seasons [3–5]. Therefore, new insights into the dynamics of influenza epidemic variants are currently needed.

In the field of avian influenza, after initiation of a vaccination program for poultry, reports of vaccine failure began emerging [6]. The acquisition of antigenic drift-related HA mutations, resulting in escape from vaccine-induced immunity, was suggested to be the most important cause of vaccine failure [6,7]. Higher mutation rates of HA genes have been reported in countries applying H5 vaccination compared to countries not applying vaccination [8]. Recently, deep sequencing showed that within-host HA genetic diversity was not significantly different between vaccinated and unvaccinated individuals [9]; however, the effect of seasonal vaccination on HA mutations remains to be clarified. In humans, drift-related mutations are considered to be primarily caused by the immune pressure elicited by natural infection. In such a scenario, the pattern of HA mutations should be independent of vaccination. We hypothesized that a key to resolving this question would be to examine genetic differences from the vaccine virus strains.

We have developed a network of physicians throughout Japan who routinely collect influenza virus samples, along with patient information including vaccination history [10–13]. In this study, we sequenced the full length of HA genes of influenza A/H3N2 viruses isolated from both vaccinated and unvaccinated patients over four consecutive influenza seasons and compared them with the yearly vaccine strains.

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2. Materials and methods

2.1. Sample collection

Nasopharyngeal swabs for influenza virus isolation were collected and vaccination history was determined from patients who had a positive result on a rapid influenza antigen test, given at one of the member clinics of our nation-wide study network of general practitioners [10–13]. Informed consent was obtained from all patients. All patients were outpatients, and this study did not include any patients with severe chronic respiratory diseases, renal diseases, liver diseases, or heart failure. All viral samples were collected before the initiation of neuraminidase inhibitors (NAIs). Background information on these patients, including their vaccination history, was also collected. In recent years, we have done surveillance studies to examine the susceptibility (50% inhibitory concentration, IC_{50}) of NAIs [14,15]. As part of our surveillance, 283 A/H3N2 virus samples were collected in the 2011–12 season and 316 in the 2012–13 season. In this study, the isolates of 181 samples (48 from the 2011–12 season, 48 from the 2012–13 season, 33 from the 2013–14 season, and 52 from the 2014–15 season) were genetically analyzed. In total, 82 isolates were obtained from vaccinated patients and 99 from unvaccinated patients. In Japan, all residents are allowed to receive seasonal influenza vaccination. All viral samples from vaccinated patients were collected more than four weeks after vaccination. A/Victoria/210/2009 (X-187), A/Victoria/361/2011 (IVR-165), A/Texas/50/2012 (X-223), and A/NewYork/39/2012 (X-233A) were used in the Japanese influenza A/H3N2 vaccines for the 2011–12, 2012–13, 2013–14, and 2014–15 seasons, respectively.

2.2. Viral RNA extraction and RT-PCR

Nasopharyngeal swabs from patients were soaked in virus transport medium and 75 μL of the medium was cultured using Madin-Darby canine kidney (MDCK) cells. Viral RNA was extracted from infected MDCK cell culture supernatants using the Maxwell 16 LEV simply RNA Cells Kit (Promega, Madison, WI). The A/H3N2 subtype was determined by PCR [16]. RT-PCR was performed using the H3N2 RNA samples. PCR primers, synthesized based on the 3′ and 5′ terminal nucleotides that are common to all human influenza A virus segments [17] were as follows: forward primer (Uni-12), 5′-ACGCGTGATCAGCAAAAG CAGG-3′ and reverse primer (Uni-13), 5′-ACGCGTGATCAGTAGAAA CAAGG-3′. The PCR consisted of 31 cycles of a denaturing step at 94 °C for 30 s, an annealing step at 57 °C for 30 s, and an extension step at 72 °C for 2 min.

2.3. Next generation sequencing

A DNA library for Illumina sequencing was prepared using the Nextera XT DNA Sample Prep kit (Illumina, San Diego, CA). Briefly, the PCR products were quantified using the Qubit dsDNA BR Assay kit (Life Technologies, Grand Isle, NY), followed by tagmentation of the input DNA (0.5 ng total). The tagmented DNA was amplified using the Nextera XT Index kit (Illumina), and then the library was cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA). Following library normalization, the amplicon library was pooled and quantified for sample loading by using the KAPA Library Quantification kit for Illumina (KAPA Biosystems, Wilmington, MA). Sequencing was conducted via a paired-end, 2 × 250 bp cycle run, using the Illumina MiSeq sequencing system and MiSeq Reagent Kit version 2 (300 Cycle) (Illumina).

2.4. Bioinformatic analysis

Data processing was performed using the pipeline prepared by Amelieff Co. Briefly, in the filtering step, reads were trimmed by removing low-quality bases and removed if they were shorter than 32 bases or if more than 80% of the read had quality lower than 20, by use of QCleaner version 3.1 (Amelieff Co., Tokyo, Japan). Filtered reads were then checked for quality by use of FastQC version 0.10.0 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Next, reads were aligned to the reference sequence A/New York/396/2005 (H3N2) (GenBank accession numbers for the eight gene segments: CY002079, CY121123, CY121122, CY121117, CY121120, CY121119, CY121118, and CY121121) using BWA (Burrows-Wheeler Aligner) version 0.7.5 (http://bio-bwa.sourceforge.net/) with MEM algorithm. The files were realigned and recalibrated with the GATK (Genome Analysis Toolkit) framework (http://www.broadinstitute.org/gatk/), including RealignerTargetCreator, IndelRealigner, CountCovariates, and TableRecalibration. Sequence variants were then detected using the GATK UnifiedGenotyper, followed by filtering for low-quality variants by use of the GATK VariantFiltration. Finally, genome sequences were constructed using the reference sequence and filtered variants. The HA amino acid (AA) sequence was deduced from the obtained nucleotide sequence.

2.5. Nucleotide sequence accession number

The sequence data from this study were deposited into the DDBJ/EMBL/GenBank nucleotide sequence databases under the following accession numbers: LC090941–LC091036, LC111574–LC111606, and LC155845–LC155896.

2.6. Phylogenetic analysis

Sequences were aligned using ClustalX version 2.1 (http://www.clustal.org/). Phylogenetic trees were constructed by the neighbor-joining method, using the MEGA 6.0 program [18].

2.7. Statistical analysis

Deviation of variables between groups was tested using the Wilcoxon rank-sum test. The other tests were performed using the chi-square test. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the JMP Pro software, version 11 (SAS Institute, Inc., Cary, NC, USA).

3. Results

Demographic data on the vaccinated and unvaccinated patients is shown in Supplementary Table 1. The age distribution (mean ± SD) was similar of the vaccinated and unvaccinated patients (25.5 ± 25.0 years vs. 23.7 ± 18.8 years, respectively; $P = 0.81$). Sample collection was done from December to January for 90.2% of the vaccinated patients and 87.9% of the unvaccinated patients, and no significant difference in sample collection timing was seen ($P = 0.75$). Viral samples were collected from all regions including northern, central, and southern Japan. The distribution of the collection areas was also similar ($P = 0.41$).

Full-length HA nucleotide sequences were obtained from 181 influenza A/H3N2 viruses isolated during four consecutive seasons (2011–15). The phylogenetic analysis showed the formation of clades for all of the seasons (Supplementary Fig. 1). As seen in the tree, there were no specific clades occupied by the viruses isolated from vaccinated patients. Thus, a phylogenetic approach did

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