Characterization of an A (H1N1)pdm09 Virus Imported from India in March 2015

Emi Takashita1, Seichiro Fujisaki1, Masayuki Shirakura1, Kazuya Nakamura1, Noriko Kishida1, Tomoko Kuwahara1, Suguru Ohmiya2, Ko Sato2, Hiroko Ito2, Fumiko Chiba2, Hidekazu Nishimura2, Shizuo Shindo3, Shinji Watanabe1, Takato Odagiri1*, and The Influenza Virus Surveillance Group of Japan**

1Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo; 2Virus Research Center, Sendai Medical Center, Miyagi; and 3Shindo Children’s Clinic, Fukuoka, Japan

Communicated by Masayuki Saijo

A large influenza A(H1N1)pdm09 outbreak occurred in India in December 2014 (1–3). Between January 1 and April 21, 2015, over 35,000 cases, and 2,335 deaths have been reported, making this one of the worst influenza outbreaks that India has experienced in recent years (3). A number of reports have suggested various reasons for this large outbreak (2,4), but detailed information about the influenza viruses causing this outbreak in India is limited.

In March 2015, we isolated an Indian-origin A(H1N1)pdm09 virus from a patient in Japan, who had visited India during the outbreak. Here, we report the characteristics of the Indian-origin virus in order to contribute to the understanding of the large outbreak. The patient was an Indian girl aged 6 years living in Fukuoka, Japan, who had visited India with her family and returned to Japan on March 9, 2015. The patient’s mother had a high fever on March 10. She was diagnosed as having influenza A virus infection, and lanaminavir was administered on March 11. Her symptoms subsequently improved. The 6-year-old patient had a high fever and headache and was diagnosed as having influenza A virus infection at Shindo Children’s Clinic on March 16. March 17 onwards, oseltamivir (2 mg/kg) was administered two times a day for 5 days, which improved the patient’s symptoms. The patient’s mother had not received an influenza vaccine, but the patient herself had been vaccinated twice, once on November 13, 2014 and once on December 20, 2014. After informed consent, a nasal swab specimen was collected before the oseltamivir treatment at Shindo Children’s Clinic. The influenza A(H1N1)pdm09 strain A/Fukuoka/SDC1/2015 was isolated from the specimen at the Sendai Medical Center.

Phylogenetic analysis of the hemagglutinin (HA) (Fig. 1A) and neuraminidase (NA) (Fig. 1B) genes of A/Fukuoka/SDC1/2015 indicated that it originated in India during the outbreak. The Indian-origin A(H1N1)pdm09 and all Indian viruses analyzed in this study belong to genetic clade 6B that contains majority of the globally circulating influenza viruses (5,6). Three amino acids characteristic of the Indian viruses causing the worst outbreak, S84N in the HA protein, and V131I and I314M in the NA protein, were detected. Further analysis of additional A(H1N1)pdm09 viruses from the Indian outbreak is required to assess the significance of these substitutions. A D222G substitution in the HA protein, that is associated with increased pathogenicity (7,8), was detected in only 1 out of 13 Indian viruses isolated in the 2014–2015 season (Fig. 1A), suggesting that the D222G substitution is unrelated to the large outbreak.

No significant antigenic differences were found between the Indian-origin A(H1N1)pdm09 and the recently circulating A(H1N1)pdm09 viruses in Japan (Table 1). They were antigenically similar to the vaccine strain A/California/07/2009. These results suggest that the influenza vaccine recommended by the World Health Organization (WHO) for the 2014–2015 northern hemisphere influenza season, was well matched to the Indian viruses causing the outbreak.

Almost all global circulating A(H1N1)pdm09 and A(H3N2) viruses possess an S31N substitution in the M2 protein, which confers resistance to M2 inhibitors. Therefore, the WHO recommends NA inhibitors for the treatment of influenza patients (9). The Indian-origin A(H1N1)pdm09 and all other Indian influenza viruses examined possessed the S31N substitution, indicating that these viruses have M2 inhibitor resistance. Since the A(H1N1)pdm09 virus began circulating globally in 2009, NA inhibitor-resistant viruses have been detected sporadically. However, community clusters of oseltamivir and peramivir cross-resistant viruses occurred in Australia in 2011, and in Japan in 2013–2014, respectively (10,11). Significant numbers of these resistant viruses were also detected in the United States and China during the 2013–2014 season (6,12,13). The resistant viruses possessed an H275Y substitution in the NA protein. The H275Y substitution or other known substitutions associated with the reduced susceptibility to NA inhibitors were not observed in the Indian-origin A(H1N1)pdm09 or any other Indian viruses strains.

We determined the susceptibility of the Indian-origin A(H1N1)pdm09 virus to currently available NA inhibitors including, oseltamivir, peramivir, zanamivir, and...
Fig. 1. Phylogenetic analysis of the hemagglutinin (HA; A) and neuraminidase (NA; B) genes of the influenza A(H1N1)pdm09 viruses: the phylogenetic trees were constructed using the MEGA6 software (14) with the neighbor-joining method. Nucleotide sequences of the Indian viruses were retrieved from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID). The open and closed circles indicate the Indian viruses isolated in the 2013–2014 and 2014–2015 influenza seasons, respectively. The amino acids are described with the H1 and N1 numbering. The A/California/07/2009 virus is used as a reference strain for numbering and substitutions of amino acids, and those are shown to the left of the nodes. The scale bar indicates the nucleotide substitutions per site. The nucleotide sequences determined in this study are available from the GISAID database. Accession numbers for the HA, NA, and M genes of A/Fukuoka/SDC1/2015 are EPI588942, EPI588941, and EPI588937, respectively.

laninamivir by using a fluorescent NA inhibition assay (11). The results are expressed as the drug concentration required to inhibit NA activity by 50% (IC50). The median and interquartile range of IC50s (nM) of A(H1N1)pdm09 viruses isolated in the 2014–2015 season in Japan to oseltamivir, peramivir, zanamivir, and laninamivir were 0.27 ± 0.14, 0.07 ± 0.02, 0.23 ± 0.12, and 0.29 ± 0.15, respectively. The IC50s of the Indian-origin A(H1N1)pdm09 virus to each drug were 0.56, 0.05, 0.28, and 0.12 nM, respectively. These results indicate that the Indian-origin virus was susceptible to all NA inhibitors. Two patients from a family cluster of this Indian influenza virus infection received oseltamivir and laninamivir treatment within 24 h of symptom onset, respectively. Their symptoms improved after administration of the drugs, suggesting that the NA inhibitors were effective against the Indian viruses that caused the large outbreak.

To assess the risk of global spread of A(H1N1)pdm09 viruses that resulted in the worst recent outbreak of influenza in India, in the following seasons, continued and strengthened surveillance of these viruses is needed to protect public health.

Acknowledgments  We thank Hiromi Sugawara, Aya Sato, Rie Ogawa, Hideka Miura, and Miki Akimoto for technical assistance and Bruce Haendel for language editing. This work was supported, in part, by Grant-in-Aid for Emerging and Reemerging Infectious Diseases from the Ministry of Health, Labour and Welfare, Japan and by JSPS KAKENH grant 26460816.

Conflict of interest None to declare.

Appendix The members of the Influenza Virus Surveillance Group of Japan are as follows: Rika Komagome (Hokkaido Institute of Public Health), Asami Ohnishi (Sapporo City Institute of Public Health), Rika Tsutsui (Aomori Prefectural Institute of Public Health and Environment), Masaki Takahashi (Research Institute for Environmental Sciences and Public Health of Iwate Prefecture), Yoko Suzuki (Miyagi Prefectural Institute of Public Health and Environment), Ayumi Nakata (Sendai City Institute of Public Health), Chihiro Shibata (Akita Prefectural Research Center for Public Health and Environment), Yoshiko Kashiwagi (Fukushima Prefectural Institute of Public Health), Shikako Hirokawa (Niigata Institute of Public Health and Environmental Sciences), Kazunari Yamamoto (Niigata City Institute of Public Health and Environment), Ikuko Doi (Ibaraki Prefectural Institute of Public Health), Fuminori Mizukoshi (Tochigi Prefectural Institute of Public Health and Environmental Sciences), Chikako Nagashima (Utsunomiya City Institute of Public Health and Environment), Hiroyuki Tsukagoshi (Gunma Prefectural Institute of Public Health and Environmental Sciences), Noriko Suzuki (Saitama Institute of Public Health and Environment Science), Atsushi Ogura (Chiba Prefectural Institute of Public Health), Ayano Mizumura (Chiba City In-
Table 1. Hemagglutination inhibition (HI) titers of the influenza A(H1N1)pdm09 viruses

| Virus strain                  | Collection date | HI titers using post-infection ferret antisera against: |
|-------------------------------|-----------------|--------------------------------------------------------|
|                               |                 | A/California/07/2009 | A/Narita/1/2009 | A/Wakayama/153/2013 |
| **Reference viruses**         |                 |                          |                          |                          |
| A/California/07/2009          | 2009/04/09      | 2,560                    | 1,280                    | 1,280                    |
| A/Narita/1/2009              | 2009/05/08      | 5,120                    | 2,560                    | 2,560                    |
| A/Wakayama/153/2013          | 2013/11/05      | 2,560                    | 2,560                    | 1,280                    |
| **Test viruses**             |                 |                          |                          |                          |
| A/Fukuoka/SDC1/2015          | 2015/03/16      | 2,560                    | 2,560                    | 2,560                    |
| A/Yamanashi/14332/2014       | 2014/12/12      | 2,560                    | 1,280                    | 1,280                    |
| A/Fukuoka-c/6/2015          | 2015/01/21      | 2,560                    | 2,560                    | 2,560                    |
| A/Oita/6/2015               | 2015/01/22      | 2,560                    | 2,560                    | 1,280                    |
| A/Fukuoka/14363/2015        | 2015/02/09      | 2,560                    | 1,280                    | 2,560                    |
| A/Ibaraki/21/2015           | 2015/02/09      | 1,280                    | 1,280                    | 1,280                    |
| A/Hyogo/3174/2015           | 2015/02/11      | 1,280                    | 2,560                    | 1,280                    |
| A/Saga/32/2015              | 2015/03/16      | 1,280                    | 2,560                    | 1,280                    |

1) HI test was carried out using turkey red blood cells according to the WHO manual for the laboratory diagnosis and virological surveillance of influenza (15).
2) A vaccine strain recommended by WHO for the 2014–2015 northern hemisphere influenza season.
3) A representative A(H1N1)pdm09 virus isolated in Japan in a pandemic period.
4) A representative A(H1N1)pdm09 virus isolated in Japan in the 2013–2014 influenza season.
5) An influenza virus studied in this study.

Fig. 1. Continued

...
REFERENCES

1. Bagchi S. India tackles H1N1 influenza outbreak. Lancet. 2015; 385:e21.
2. Mishra B. 2015 Resurgence of Influenza A (H1N1) 09: Smoldering pandemic in India? J Glob Infect Dis. 2015;7:56-9.
3. D'Silva J. Swine flu: how well did India respond? BMJ. 2015;350:h2286.
4. Tharakaraman K, Sasisekharan R. Influenza surveillance: 2014–2015 H1N1 “swine”-derived influenza viruses from India. Cell Host Microbe. 2015;17:279-82.
5. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2015–2016 northern hemisphere influenza season. Wkly Epidemiol Rec. 2015;90:97-108.
6. Takashita E, Meijer A, Lackenby A, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. Antiviral Res. 2015;117:27-38.
7. Chutinimitkul S, Herfst S, Steel J, et al. Virulence-associated substitution D222G in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. J Virol. 2010;84:11802-13.
8. Liu Y, Childs RA, Matrosovich T, et al. Altered receptor specificity and cell tropism of D222G hemagglutinin mutants isolated from fatal cases of pandemic A(H1N1) 2009 influenza virus. J Virol. 2010;84:12069-74.
9. WHO. WHO guidelines for pharmacological management of pandemic (H1N1) 2009 influenza and other influenza viruses. Available at <http://www.who.int/csr/resources/publications/swineflu/h1n1_use_antivirals_20090820/en/>. Accessed October 6, 2015.
10. Hart AC, Hardie K, Wilson NJ, et al. Characteristics of a widespread community cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza in Australia. J Infect Dis. 2012;206:148-57.
11. Takashita E, Kiso M, Fujisaki S, et al. Characterization of a large cluster of influenza A(H1N1)pdm09 viruses cross-resistant to oseltamivir and peramivir during the 2013–2014 influenza season in Japan. Antimicrob Agents Chemother. 2015;59:2607-17.
12. Okomo-Adhiambo M, Fry AM, Su S, et al. Oseltamivir-resistant influenza A(H1N1)pdm09 viruses, United States, 2013-14. Emerg Infect Dis. 2015;21:136-41.
13. Huang W, Li X, Cheng Y, et al. Characteristics of oseltamivir-resistant influenza A (H1N1) pdm09 virus during the 2013–2014 influenza season in Mainland China. Virol J. 2015;12:96.
14. Tamura K, Stecher G, Peterson D, et al. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725-9.
15. WHO. Manual for the laboratory diagnosis and virological surveillance of influenza. Available at <http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf>. Accessed October 6, 2015.