JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1983, p. 425–427
0095-1137/83/080425-03$02.00/0

Vol. 18, No. 2

Direct Isolation of H1N2 Recombinant Virus from a Throat Swab of a Patient Simultaneously Infected with H1N1 and H3N2 Influenza A Viruses

FUMIO NISHIKAWA* AND TEIJI SUGIYAMA

Department of Virology and Rickettsiology, National Institute of Health, Kamiosaki, Tokyo 141, Japan

Received 22 February 1983/Accepted 18 April 1983

Two H1N2 recombinant viruses were isolated by a plaquing method from a throat swab of a patient who was simultaneously infected with H1N1 and H3N2 influenza viruses during the Tokyo epidemic of 1981. This is the first direct evidence that recombination of influenza viruses occurred in the human body.

Since H1N1 influenza virus reappeared in 1977, there have been several reports showing that two subtypes (H1N1 and H3N2) of influenza A viruses were prevalent during the same periods and in the same areas (1, 3, 6, 7). Several cases of simultaneous infection with these two subtype viruses in one patient were observed in some countries, and attempts were made to isolate recombinant virus. Recombinant viruses were found, but in all cases, they were isolated from allantoic fluids after throat swabs of the patients were inoculated into eggs (4, 9, 10). Thus, the possibility could not be ruled out that the patients' throat swabs contained only two subtypes of parent viruses, and the recombinant viruses were formed in the eggs, as those reports discussed. We observed two cases of double infection with two subtypes of influenza viruses in 1981. This report describes the direct isolation of the recombinant virus from the throat swabs of one of the patients.

The plaque method for virus isolation was carried out with MDCK cells. The virus was grown in chicken embryo allantoic cavity. The hemagglutination inhibition test was done by using type-specific rabbit serum against A/Kumamoto/37/79 (H1N1) and A/Bangkok/1/79 (H3N2), and the neuraminidase inhibition test was conducted by the World Health Organization method after the virus was treated with Triton X-100 (2, 8).

Influenza H1N1, H3N2, and B-type viruses were prevalent from February to April in 1981 in southeast Tokyo. Of 88 patients with respiratory illnesses in this area, 47 were demonstrated to be infected with influenza viruses by serological tests or virus isolation or both. Among them, two brothers (patients no. 81-43, 5 years old, and no. 81-48, 3 years old) fell ill at a 2-day interval in the middle of February. Throat swabs were collected from each 1 day after onset, and viruses were isolated from each specimen in monkey kidney primary cell tube cultures. The virus 81-48 was identified as H3 influenza virus by the hemagglutination inhibition test. The virus 81-43 was difficult to identify because of its inability to be inhibited in hemagglutination inhibition tests with H1, H3, or B-type-specific antiserum, but it was identified as H3 virus after one more passage by limiting dilution. Paired sera of one patient (no. 81-48) showed antibody rises to both H1 and H3 antigens by the hemagglutination inhibition test (serum of patient 81-43 could not be collected). Then an attempt was made to examine their throat swabs in more detail.

Each throat swab was inoculated into MDCK cells to isolate virus by the plaque method. Of 30 plaques collected from the 81-43 throat swab, 21 clones grew in embryonated eggs. Twenty-seven plaques were isolated from the 81-48 throat swab, and 17 of them could be passaged by egg inoculation. Identifications of the subtypes of hemagglutinin and neuraminidase were done for these egg-grown, cloned viruses. Table 1 gives an example of serological characterization of isolated clones. Nineteen clones from patient 81-43 were identified as H1N1, and two clones were H1N2 recombinant viruses (Table 1). In this examination, numbers of plaques formed at each dilution of the swab fluid (81-43) were as follows: at 10^0, 27, 31, 23; at 10^{-1}, 9, 8, 9, 5; at 10^{-2}, 2, 3, 2, 0. Recombinant viruses of 81-43-02-2 and 81-43-1-2 (Table 1) were recovered from clearly isolated plaques formed at 10^0 and 10^{-1} dilutions of the swab fluid, respectively. The virus of 81-43-02-2 was further tested by plaque formation without growth in any hosts, and four plaques collected were all H1N2 recombinant viruses. However, H3N2 and H3N1 recombinants were not isolated. On the other hand, one H1N1 and 16 H3N2 viruses were isolated from patient 81-48, but no recombinant virus was obtained (Table 1).

From these results, it was demonstrated by

TABLE 1. Hemagglutinin and neuraminidase subtype identification of cloned virus[a]

Clone	HI titer with antiserum to:		NI titer with antiserum to:		Composition
	A/Kumamoto/ 37/79 (H1)	A/Bangkok/ 1/79 (H3)	A/Kumamoto/ 37/79 (N1)	A/Bangkok/ 1/79 (N2)	
81-43-					
01-2	512	<128	160	<4	H1N1
02-1	1,024	<128	250	<4	H1N1
02-2	512	<128	<4	100	H1N2
0-3	512	<128	210	<4	H1N1
0-6	256	<128	250	<4	H1N1
0-7	256	<128	250	<4	H1N1
1-2	256	<128	<4	180	H1N2
1-3	256	<128	250	<4	H1N1
1-4	256	<128	160	<4	H1N1
81-48-					
0-1	<128	≥1,024	<4	105	H3N2
0-2	<128	≥1,024	<4	80	H3N2
0-3	<128	≥1,024	<4	105	H3N2
0-4	<128	≥1,024	<4	140	H3N2
0-5	<128	≥1,024	<4	80	H3N2
0-6	512	<128	100	<4	H1N1
0-7	<128	≥1,024	<4	105	H3N2
0-8	<128	≥1,024	<4	80	H3N2
Kumamoto	1,024	<128	160	<4	H1N1
Bangkok	<128	4,096	<4	150	H3N2

[a] Abbreviations: HI, hemagglutination inhibition; NI, neuraminidase inhibition.
[b] Recombinant virus.

virus isolation with monkey kidney primary and MDCK cells that the two siblings were simultaneously infected with H1 and H3 subtypes of influenza viruses. Recombinant viruses were isolated from one of their throat swabs directly by the plaque method without passage through any laboratory hosts. Therefore, it is strongly suggested that the recombination of influenza virus occurred in the human body when two subtypes of viruses grew simultaneously in this patient.

In the throat swab we found H1N2 viruses but could not find H3N1 virus, whereas Yamane et al. (10) and Kendal et al. (4) obtained only an H3N1 recombinant, and Sakoh et al. (9) isolated six clones of H3N1 and only one clone of H1N2 recombinant viruses from egg-passaged throat swabs of patients suffering from double infections. Two types of recombinants (H1N2 and H3N1) may be formed in the human body, but we found only one combination in our experiment; perhaps H3N1 virus grows more easily in embryonated eggs than does H1N2 virus.

Since the winter of 1977–1978, influenza H1N1 and H3N2 viruses were prevalent in some parts of the world at the same periods. Young and Palese (11) found recombinant virus in which hemagglutinin, neuraminidase, matrix, and nonstructural protein genes were derived from H1N1 virus, and polymerase 1, 2, 3 and nucleoprotein genes originated from H3N2 parent in the winter of 1978–1979 in the United States. Nakajima et al. (5) demonstrated that this type of recombinant virus was prevalent all over the world. It is not unlikely that such recombinational events took place in the human body, but direct evidence of this has not been shown. The present studies confirmed the origin of each gene for the cloned viruses obtained directly from the throat swab of a patient coinfected with two subtypes. It is puzzling that viruses with recombination in hemagglutinin or neuraminidase genes, such as H1N2 or H3N1, were not prevalent in nature, whereas H1N1 recombinant virus in which polymerase and nucleoprotein genes were derived from H3N2 virus was prevalent.

We thank H. Abe and M. Akita, Pediatrics of Social Insurance Associations Kamata General Hospital, for their cooperation in the collection of experimental materials and epidemiological data.

LITERATURE CITED

1. **Antonova, V., G. G. Khokhlova, and V. M. Zhdanov.** 1978. An unusual influenza situation in 1977–78. W.H.O. Chron. **32**:469–471.
2. **Aymard-Henry, M., M. T. Coleman, W. R. Dowdle, W. G. Laver, G. C. Schild, and R. G. Webster.** 1973. Influenza virus neuraminidase and neuraminidase-inhibition test procedures. Bull. W.H.O. **48**:199–202.
3. **Kendal, A. P., J. M. Joseph, G. Kobayashi, D. Nelson, C. R. Reyes, M. R. Ross, J. L. Sarandria, R. White, D. F. Woodall, G. R. Noble, and W. R. Dowdle.** 1979. Laboratory-based surveillance of influenza virus in the United

States during the winter of 1977–1978. I. Periods of prevalence of H1N1 and H3N2 influenza A strains, their relative rates of isolation in different age groups, and detection of antigenic variants. Am. J. Epidemiol. **110**:449–461.

4. **Kendal, A. P., D. T. Lee, H. S. Parish, D. Raines, G. R. Noble, and W. R. Dowdle.** 1979. Laboratory-based surveillance of influenza virus in the United States during the winter of 1977–1978. II. Isolation of a mixture of A/Victoria- and A/USSR-like viruses from a single person during an epidemic in Wyoming, USA, January 1978. Am. J. Epidemiol. **110**:462–468.
5. **Nakajima, S., N. J. Cox, and A. P. Kendal.** 1981. Antigenic and genomic analyses of influenza A(H1N1) viruses from different regions of the world, February 1978 to March 1980. Infect. Immun. **32**:287–294.
6. **Nakajima, S., K. Nakajima, Y. Takeuchi, and A. Sugiura.** 1980. Influenza surveillance based on oligonucleotide mapping of RNA of H1N1 viruses prevalent in Japan, 1978–1979. J. Infect. Dis. **142**:492–502.
7. **Pereira, M. S., and P. Chakraverty.** 1982. Influenza in the United Kingdom 1977–1981. J. Hyg. **88**:501–512.
8. **Russ, G., E. Vareckova, and B. Styk.** 1974. Steric effects in the reaction of influenza virus neuraminidases with antibodies. Acta Virol. (Engl. Ed.) **18**:299–306.
9. **Sakoh, M., T. Nakayama, K. Aihara, T. Oka, S. Nonaka, K. Nerome, A. Oya, and K. Ueda.** 1980. Subtypes H1N1 and H3N2 of influenza virus isolated from the same patient. Virus **30**:33–40. (In Japanese.)
10. **Yamane, N., J. Arikawa, T. Odagiri, N. Sukeno, and N. Ishida.** 1978. Isolation of three different influenza A viruses from an individual after probable double infection with H3N2 and H1N1 viruses. Jpn. J. Med. Sci. Biol. **31**:431–434.
11. **Young, J. F., and P. Palese.** 1979. Evolution of human influenza A viruses in nature: recombination contributes to genetic variation of H1N1 strains. Proc. Natl. Acad. Sci. U.S.A. **76**:6547–6551.