—Preliminary Note—

Immunogenicity of inactivated influenza vaccine containing A/Niigata/102/81 (H3N2)

Increase of HAI antibodies to different variants of the subtype

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Inactivated influenza vaccine¹⁾ has been widely used in some countries in the Temperate Zone, but the virus strain contained in it should be deliberately chosen in each subtype because of the continuous antigenic drift of the hemagglutinin (HA) of the virus. The A/Niigata/102/81-variant of Hong Kong subtype (H3N2) of influenza virus was recommended as the vaccine strain by the Japanese Ministry of Health and Welfare in 1982, because it was first isolated in Thailand as A/Bangkok/212/81 in January, in Japan in March^{2~7)} and was predicted to prevail in Japan in the winter season of 1982-1983. Although the A/Kyoto/C-1/81-variant (H3N2) of the virus, which was also first isolated in Thailand as A/Bangkok/137 and 138/80 in August and in Japan in February^{2~7)}, was predicted to be an epidemic virus in the winter of 1982-1983, it was not qualified to be the vaccine-virus because of the relatively poor growth in developing hen's eggs. Therefore, it was expected that the vaccine containing A/Niigata/102/81 has the immunogenicity against A/Kyoto/C-1/81 and other variants of the Hong Kong subtype. In addition, according to the theory of original antigenic sin, the immunological memory of a past antigenic stimulus of a variant of influenza virus could affect the immune response of humans against a later antigenic stimulus of another variant. In this report, hemagglutination inhibiting (HAI) titers against A/Bangkok/1/79, A/Niigata/102/81, and A/Kyoto/C-1/81 viruses in serum samples from volunteers immunized with the inactivated vaccine containing A/Niigata/102/81 were studied.

The ether tween-80-split vaccine^{8,9)} containing 600 CCA/ml of A/Niigata/102/81 virus HA was produced by Chiba Prefectural Serum Institute. Briefly, the virus in chorioallantoic fluid of infected developing hen's eggs was purified by fractional and Spinco-centrifugation after the adsorption-elution from barium sulfate. The purified virus particles were split

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with ether and Tween-80, and the water layer was stocked at 4°C after adding formalin.

Volunteers were 10 staff members, 25 to 27 years of age, of the Institute for Gerontology, Nippon Medical School (IGN), and 18 students, 18 to 33 years of age, of the Kashima White Cross Nursing School, Ibaraki (KNS). Each group included both sexes. Differences of HAI titers were studied by the Student's *t* test. A 0.5 ml dose of the vaccine was subcutaneously injected twice with an interval of 14 days in July, 1983. As control, 7 students of KNS were not immunized; and 2 students received only the first dose of the vaccination. No obvious ill effect was observed. Blood samples were collected before the first dose and 14 days after the second dose.

HAI titers of serum samples were determined after treatment with RDE (Takeda Pharm. Co.) against egg-grown A/Bangkok/1/79, A/Niigata/102/81, and A/Kyoto/C-1/81 viruses by using chicken blood cells¹⁰⁾.

Table 1 Geometric mean HAI titers of volunteers before immunization

Antigen	HAI titer ^{a)} ±SD		
	KNS ^{b)} (18)	IGN ^{b)} (10)	Total (28)
A/Bangkok/1/79	8.00±1.85	6.10±1.20	7.32±1.87
A/Niigata/102/81	5.28±1.32	4.50±1.58	5.00±1.44
A/Kyoto/C-1/81	5.00±1.28	4.20±0.63	4.71±1.15

a) HAI titer are expressed as log₂ reciprocal numbers of serum dilution which completely inhibited the hemagglutination with 4 HA doses of the antigen. log₂ HAI titer of less than 1:32 was referred to as 4.

b) KNS, Kashima White Cross Nursing School. IGN, Institute for Gerontology, Nippon Medical School. Number in parentheses is case number.

HAI titers before the immunization are shown in **Table 1**, which describes that geometric mean (GM) HAI titer against A/Bangkok/1/79 was higher than both of A/Kyoto/C-1/81 (*p*<0.001) and A/Niigata/102/81 (*p*<0.05) in each group and in total volunteers. No significant difference was observed between HAI titers against A/Kyoto and A/Niigata. These findings suggest that A/Bangkok/1/79-like virus had been more prevalent than the other two viruses in Kanto area until June, 1983. GM HAI titer of the KNS group was higher than the IGN group, if A/Bangkok (*p*<0.01) or A/Kyoto (*p*<0.05) was used as the antigen. No significant difference (*p*>0.1) between the two groups was observed against A/Niigata. These results may be ascribed to the difference in job between the two groups; i.e., KNS students were assistant nurses, whereas IGN members were histological technicians.

HAI titers of the volunteers before and after the immunization are shown in **Fig. 1**. As the first sera were collected in July and the second in August, increase of the antibody titer to each of the H3N2 viruses was not observed in volunteers who did not receive the immunization. However, increase of HAI titers of volunteers who received two shots of the vaccine was observed against all of the H3N2 viruses tested. Increase of the HAI titer to A/Niigata after the immunization was more significant than that to A/Bangkok in the KNS group (*p*<0.05) and in total volunteers (*p*<0.01), but not in the IGN group

($p>0.1$). This is due to a very low anti-A/Niigata HAI titer of the IGN group before the immunization. Immunogenic effect of the inactivated influenza vaccine will be enhanced by the preexisting immunity. Increase of the HAI titer to A/Niigata was more significant than that to A/Kyoto in total volunteers ($p<0.01$), but not in each of the separate groups ($p>$

0.05) because of the small case number of each group. This also suggests the antigenic difference between A/Kyoto and A/Niigata. Significant difference was not observed between increases of the HAI titers to A/Bangkok and those to A/Kyoto after the immunization.

From these results it will be concluded that: ① A/Bangkok/1/79-like virus had been more prevalent than A/Niigata/102/81- or A/Kyoto/C-1/81-like virus until June, 1983. ② Before the immunization, HAI titers against A/Bangkok as well as A/Kyoto were higher in the KNS group (assistant nurses) than in the IGN group (histological technicians), but those against A/Niigata were not different between the two groups. ③ Immunization with a split vaccine containing A/Niigata/102/81 virus HA increased the HAI titers against A/Niigata, A/Bangkok, and A/Kyoto. ④ Increase of the HAI titers to A/Niigata with the immunization was most significant among the three H3N2 viruses tested, but a basic immunity was necessary to definitely raise the HAI titers.

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