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SHORT COMMUNICATION

SEROLOGICAL STUDIES ON VOLUNTEERS INOCULATED EXPERIMENTALLY WITH A DENGUE VIRUS STRAIN IN 1943

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In 1943, a large dengue epidemic occurred in the Osaka district and several samples of dengue virus were isolated from patients with dengue fever by workers in this Institute. These were inoculated into human volunteers to confirm that they were dengue virus. In the present study, serum samples were collected from the volunteers who had been inoculated with dengue virus and were examined serologically. In the neutralization test, all the sera showed a higher titer against dengue type 1 virus (DEN-1) than against the other three types of dengue virus, indicating that the virus strain isolated in 1943 was DEN-1.

One of the largest dengue epidemics in this century in the world occurred in Japan from 1942 to 1945 (Schlesinger, 1977). The epidemic expanded from the Nagasaki-Sasebo area to Osaka-Kobe district (Hotta, 1952). Since then, no epidemic of dengue fever has occurred on the Main Islands of Japan. When dengue fever occurred in Osaka in 1943, several workers in this Institute tried to isolate dengue virus from patients (Taniguchi, 1943; Taniguchi et al., 1951). They used monkeys and mice for isolation of dengue virus from sera of patients with dengue fever. As

these animals did not show any typical signs of infection, these workers then inoculated the dengue virus in sera of infected monkeys or brain homogenates of infected mice into volunteers who were staff and medical students of Osaka University. Most of the volunteers inoculated developed typical symptoms of dengue fever. This was one of the first experiments in which dengue virus was isolated from a patient with dengue fever. At the time of this work, however, the virological properties of dengue virus were not understood well and so virological and serological investigations were incomplete. Moreover, all the specimens of isolated dengue virus were lost during the confusion of World War II. Therefore, we tried to measure the antibody titers in

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TABLE 1. *N* and HI antibody titers against four types of dengue virus and JE virus.

Serum No.	N titer					HI titer				
	DEN-1	DEN-2	DEN-3	DEN-4	JE	DEN-1	DEN-2	DEN-3	DEN-4	JE
1. H.S.	57 (50)	<10	35	<10	250	<10	<10	<10	<10	<10
2. Y.O.	46 (29)	<10	33	<10	100	10	<10	10	10	20
3. T.F.	135 (370)	21	35	<10	25	20	10	10	10	<10
4. S.M.	470 (350)	74	47	<10	400	40	20	20	20	20
5. T.K.	550 (740)	250	135	20	100	40	20	40	20	20
6. K.S.	<10	<10	<10	<10	700	<10	<10	<10	<10	<10
7. S.I.	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
8. H.A.	<10	<10	<10	<10	220	<10	<10	<10	<10	<10
9. K.F.	<10	<10	<10	<10	700	<10	<10	<10	<10	20
10. T.F.	50(<10)	490	56	<10	510	40	40	40	80	80
11. Y.O.	35(<10)	<10	11	16	145	10	<10	10	20	20

N antibody titers against the Mochizuki strain of DEN-1 are shown in parentheses.

sera of the volunteers retrospectively and so to identify the serotype of the infecting dengue virus.

We collected blood samples from five persons who had been volunteers at that time. These persons had been inoculated with the dengue virus strain at various passage levels in monkeys or mouse brain. For reference, blood samples were also taken from the authors of this paper, who are medical students or doctors. Sera were separated from clotted blood and stored at -20°C for tests. The viruses used for serological tests were prototype dengue viruses, type 1 (DEN-1) Hawaiian strain, type 2 (DEN-2) New Guinea B strain, type 3 (DEN-3) H-87 strain and type 4 (DEN-4) H-241 strain, and prototype Japanese encephalitis (JE) virus, Nakayama strain. The Mochizuki strain of DEN-1, which had been isolated from a patient with dengue fever during the epidemic in Nagasaki (Hotta, 1952), was also used in the neutralization (N) test. Serum antibodies against four serotypes of dengue virus and JE virus were investigated by N and hemagglutination inhibition (HI) tests. Before the N test, sera were heat-inactivated at 56°C for 30 min. N tests were performed by the focus reduction method us-

ing the PAP staining technique (Okuno et al., 1978).

The N and HI antibody titers of test sera are shown in Table 1. Serum numbers 1 to 5 represent numbers of volunteers, and those from 6 to 11 represent numbers of sera of medical students at Osaka University (No. 6-8) and staffs at this Department (No. 9-11). Four of five sera from volunteers showed broadly reactive antibodies with low titers against dengue and JE viruses in HI tests. The serotypes of the infecting dengue virus could not be decided by the tests. However, the serotype could be determined by N tests, since the titers of the volunteers were highest against DEN-1 of the four serotypes of dengue virus. Serum samples from volunteers gave similar N titers against the Mochizuki strain to those against the prototype DEN-1, while samples No. 10 and 11 gave negative titers against the Mochizuki strain. T. F. had stayed in Thailand for several years and Y. O. had accidentally been infected with a dengue virus strain isolated in Thailand in 1978 (Okuno et al., 1982).

The present study clearly showed that the virus isolated from a patient with dengue fever by Taniguchi et al. had been DEN-1. The

accompanying paper shows that the highest titers in sera of aged residents in Osaka were against DEN-1. These observations indicate that the dengue epidemic that occurred in Osaka in 1943 was due to DEN-1.

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