

## Presence of Neutralizing Antibody against the 229E Strain of Coronavirus in the Sera of Residents of Sendai

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(Received for publication, December 7, 1970)

Coronaviruses of man, recently recovered from patients with colds using tissue culture or organ culture techniques [7, 10], have emerged as one of the major etiological agents of upper respiratory illness [3, 5, 10]. These agents were all shown to possess a similar morphology [1, 6] which resembled that of avian infectious bronchitis virus (IBV) [2] and mouse hepatitis virus (MHV) [9]. The virus particles were spherical or oblong, measured 100 to 160  $m\mu$  in diameter, and surrounded by a fringe of club- or pear-shaped surface projections, about 10  $m\mu$  wide at the outer edge, and approximately 20  $m\mu$  in length. In 1966, Hamre and Procknow described the recovery of 229E strain, one of the coronaviruses, from several students with colds using standard tissue culture techniques [4]. Chanock and his associates [5, 8] confirmed 229E virus infections by virus isolation and serologic test in patients with upper respiratory illness during the winter months in 1964 and 1967. According to the report by Tyrrell group, about 30% of residents of England possessed neutralizing antibody against the 229E virus, suggesting that 229E virus probably circulates to a significant extent there [3].

This short communication describes the presence of neutralizing antibody against the 229E virus in the sera of residents of Sendai. This is the first report demonstrating the presence of coronavirus infection in Japan.

The 229E strain was supplied from Dr. Kapikian, NIH, Bethesda, as lyophilized WI-38 cell culture fluid. It was passed several times in WI-38 and human embryonic kidney (HEK) cells in our laboratory. Sera were collected over a period from January 1969 to September 1970 from 139 subjects living in the city of Sendai, and were tested by neutralization test procedures. Neutralization tests were performed by the conventional test tube method using HEK cell monolayers. Equal volumes of 32TCD<sub>50</sub> virus suspension diluted with Eagle's minimal essential medium and 2-fold serial dilutions of serum in Earle's balanced salt solution were incubated at room temperature for 2 hr.

All sera were inactivated at 56 C for 30 min before test. After incubation, 0.2 ml of each mixture was inoculated into HEK cells, and the tubes were incubated on rotating drum at 33 C. The cultures were examined for CPE at 2- to 3-day intervals

Table 1. Titer of NT antibody against 229E virus in residents of Sendai

Titer of NT antibody	No. of tested
$\leq 8$	127
1 : 8	8
1 : 16	3
1 : 32	1
Total	139

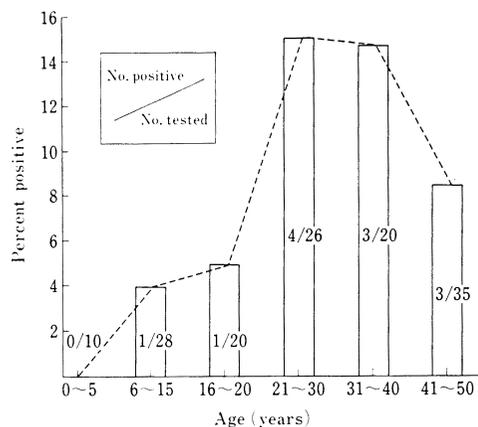


Fig. 1. Age distribution of NT antibody against 229E virus in residents of Sendai (1969-1970).

for a week. As shown in Table 1, 12 sera neutralized the virus at 1:8 or higher dilutions. Judging from the positive neutralizing antibody level (1:10) proposed by Tyrrell et al. [3], 8.6% of residents of Sendai, so far tested, were shown to possess neutralizing antibody against 229E virus. As shown in Fig. 1, about 4%, 15% and 9% in the age groups, 6-20, 21-40, 41-50 years, respectively, were found to possess detectable amount of antibody, but none of the children, 0-5 years of age, had antibody.

These observations indicate the dissemination of 229E virus or antigenically related virus in Sendai. The antibody incidence, however, was very low in both adults and children as compared with that previously reported in England [3] and the United States [5], a fact indicating a very limited virus dissemination in Sendai.

## REFERENCES

- [1] Becker, B. A., McIntosh, K., Dees, H. J., and Chanock, R. M. 1967. Morphogenesis of avian infectious bronchitis virus and a related human virus (strain 229E). *J. Virology*, Oct. 1967: 1019-1027.
- [2] Berry, D. M., Cruickshank, J. G., Chu, H. P., and Wells, R. J. H. 1964. The structure of infectious bronchitis virus. *Virology* **23**: 403-407.
- [3] Bradburne, A. F., Bynoe, M. L., and Tyrrell, D. A. J. 1967. Effect of a "new" human respiratory virus in volunteers. *Brit. Med. J.* **1**: 767-769.
- [4] Hamre, D., and Procknow, J. J. 1966. A new virus isolated from the human respiratory illness. *Proc. Soc. Exp. Biol. Med.* **121**: 190-193.
- [5] Kapikian, A. Z., James, H. D., Kelly, S. J., Dees, J. H., Turner, H. C., McIntosh, K., Kim, H. W., Parrott, R. H., Vincent, M. M., and Chanock, R. M. 1969. Isolation from man of "avian infectious bronchitis virus-like" virus (coronavirus) similar to 229E virus, with some epidemiological observations. *J. Infect. Dis.* **119**: 282-290.
- [6] Letters to Nature. 1968. *Nature* **220**: 650.
- [7] McIntosh, K., Dees, J. H., Becker, W. B., Kapikian, A. Z., and Chanock, R. M. 1967. Recovery in tracheal organ culture of novel viruses from patients with respiratory disease. *Proc. Nat. Acad. Sci. U. S.* **57**: 933-940.
- [8] McIntosh, K., Kapikian, A. Z., Turner, H. C., Hartley, J. W., Parrott, R. H., and Chanock, R. M. 1970. Seroepidemiologic studies of coronavirus infection in adults and children. *Amer. J. Epidemiol.* **91**: 585-592.
- [9] Tyrrell, D. A. J., and Almedia, J. D. 1967. Direct electron-microscopy of organ cultures for the detection and characterization of viruses. *Arch. Ges. Virusforsch.* **22**: 417-425.
- [10] Tyrrell, D. A. J., and Bynoe, M. L. 1965. Cultivation of a novel type of common cold virus in organ culture. *Brit. Med. J.* **1**: 1467-1470.