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STUDY OF THE MECHANISM OF NEUTRALIZATION OF FLAVIVIRUS  
BY MONOCLONAL ANTIBODIES AGAINST NEGISHI VIRUS

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The mechanism of neutralization of Flavivirus was studied using monoclonal antibodies against Negishi virus in the neutralization (N) test and the kinetic neutralization test.

The results were summarized as follows :

- 1) Ten monoclonal antibodies fell into three groups according to their reaction patterns in the hemagglutination-inhibition (HI) and N tests : group 1, two monoclonal antibodies, which were positive in both HI and N tests ; group 2, seven monoclonal antibodies, which were positive only in the HI test, groups 3, one monoclonal antibody, which was positive only in the N test.
- 2) The neutralizing activity of some monoclonal antibodies, which had high HI activity, was enhanced by washing cells after adsorption of virus-antibody complex.
- 3) Ten monoclonal antibodies of the three groups were classified into seven types according to the effects of washing cells after adsorption and the effects of anti-mouse IgG rabbit serum.
- 4) In the kinetic N assay, the kinetic curve had two phases, initial and equilibrium. Initial phase showed an immediate and rapid decrease of surviving virus, and in equilibrium phase, decrease of surviving virus was hardly detected. The initial slope of the kinetic curve depended on the concentration of monoclonal antibodies ; however, the surviving fraction in equilibrium phase, which had a high concentration of antibodies, was always constant, showing no dependence on the concentration of antibodies.
- 5) Double-kinetic N assay was performed by adding an equal amount of second monoclonal antibody after the first kinetic assay was completed. When the second monoclonal antibody was the same as the first one, neutralization did not proceed further. However, when the second monoclonal antibody was different from the first one, three kinetic patterns emerged : i) neutralization proceeded again ; ii) neutralization did not proceed further ; and iii) neutralization was enhanced.
- 6) The mixtures of several monoclonal antibodies remarkably enhanced neutralization in the kinetic assay. The results suggested that effective neutralization of Flavivirus required antibody binding to more than one antigenic determinant, and that Flavivirus neutralization was caused by a multi-hit mechanism.