

Classification of Inoue-Melnick Virus into Three Antigenic Types

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Antigenic analysis of Inoue-Melnick virus, formerly called SMON virus, showed that isolates from patients with subacute myelo-optico-neuropathy (SMON) in Japan, thus far tested, belonged to a single antigenic type (type 1). However, Inoue-Melnick virus isolates obtained so far in the United States chiefly from patients with multiple sclerosis could be classified into three types: type 1, type 2, and an intermediate type. At the present state of knowledge, the virus has not been proven to be the causative agent of disease, hence its provisional designation as IMV.

A new virus, provisionally termed SMON, was isolated from stools and cerebrospinal fluid (CSF) of patients with subacute myelo-optico-neuropathy (SMON) in Japan (1). As reported previously, antisera against different isolates neutralized all other strains obtained from CSF of SMON patients (3). Over 25 isolates have been made in Japan. Over the past 3 years more than 40 viruses antigenically related to SMON virus were isolated in the United States from CSF chiefly from patients with multiple sclerosis but also from patients with other chronic central nervous system diseases (2; J. L. Melnick, S. Wang, E. Seidel, G. Muchnik, L. B. Zhang, and R. Lanford, submitted for publication). Antisera to some of the isolates were prepared, and one (Baylor-6) did not show uniform reciprocal cross-neutralization with SMON reagents. This finding led to more detailed studies. We present here the antigenic classification and the results of cross-neutralization of virus isolates from Japan and the United States. We decided to call SMON virus and the related agents Inoue-Melnick virus (IMV) to avoid implicating them as causative agents of disease, particularly in the United States. Because these viruses are isolated so frequently, they deserve more intensive study.

Human diploid MRC-5 cells were used for virus assay as previously described (2; Melnick et al., submitted for publication). Tube cultures of MRC-5 cells between passages 30 and 38 were used for assay. After 3 days of cultivation in a stationary position, the growth medium was removed, and to each tube was added 1 ml of maintenance medium consisting of Eagle minimum essential medium (powder-filtrate type; GIBCO Laboratories, Grand Island, N.Y.) plus 2% inactivated fetal calf serum and 0.1% NaHCO₃. The virus suspension made in Eagle minimum essential medium (pH 6.5) was inoculated in a volume of 0.1 ml per tube. The tubes were incubated at 36 to 37°C in a roller apparatus for 6 days, after which the final readings were made.

Strain SMON-S, isolated from stools, and strains SMON-W and SMON-A, isolated from CSF of patients with SMON in Japan, were used. An additional 25 isolates from CSF of patients with SMON in different parts of Japan were also tested. Strain Baylor-1, isolated from CSF of a patient with amyotrophic lateral sclerosis in the United States, and strains Baylor-6, -9, -12, and -24, isolated from CSF of patients with multiple sclerosis in the United States, were used.

Antisera against strains SMON-S, -W, and -A and Baylor-6 and -12 were prepared in rabbits by repeated intravenous and subcutaneous injections of virus-infected culture fluid mixed with Freund complete adjuvant. Antiserum against strain Baylor-9 was from a horse injected intraspinally with the virus.

Serum was inactivated at 56°C for 30 min and diluted in twofold steps with Eagle minimum essential medium (pH 6.5). To each dilution was added an equal volume of a virus suspension containing 100 50% tissue culture infective doses per 0.1 ml, and these mixtures were incubated at 36°C for 1 h. Finally, 0.2 ml of each mixture was inoculated into three cell culture tubes. Inoculated tubes were incubated at 36 to 37°C in roller drums for 6 days.

As shown in Table 1, strains SMON-S, -W, and -A showed uniform reciprocal cross-neutralization with strain-specific antisera, but strain Baylor-6 was not neutralized by antisera against strains SMON-S, -W, or -A. Antiserum against strain Baylor-6 neutralized strains SMON-S, -W, and -A, but the ratios of heterologous to homologous neutralization titers were less than 1:10. We then tested 25 additional isolates from Japan against SMON-S and Baylor-6 antisera, and the results indicated that all 25 isolates had the same antigenicity as strain SMON-W.

Table 2 shows that antisera against strains SMON-S and -W did not neutralize strains Baylor-6 and -24 but that antiserum against strain Baylor-6 neutralized strains SMON-W and Baylor-1, -6, -9, -12, and -24. Strain Baylor-12 behaved precisely as did strain SMON-W; it showed uniform reciprocal cross-neutralization with strain SMON-W and only one-way cross-neutralization with strains Baylor-6 and -24. Strains Baylor-1 and -9 showed intermediate antigenicity between strains SMON-W and Baylor-6, whereas antiserum against strain Baylor-9 neutralized all strains equally.

Based on the data given above, the IMV isolates were classified by the ratios of heterologous to homologous neu-

TABLE 1. Antigenic analysis of IMV isolated in Japan

| Virus strain tested | Neutralization titer with indicated antiserum ^a | | | |
|---------------------|--|--------|--------|----------|
| | SMON-S | SMON-W | SMON-A | Baylor-6 |
| SMON-S | 10,000 | 4,500 | 4,500 | 180 |
| SMON-W | 10,000 | 5,000 | 5,000 | 220 |
| SMON-A | 10,000 | 5,000 | 5,000 | 220 |
| Baylor-6 | <25 | <25 | <25 | 2,500 |

^a Expressed as the 50% endpoint of serum dilution neutralizing 100 50% tissue culture infective doses of virus.

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TABLE 2. Antigenic classification of IMV isolated in the United States

| Virus strain tested | Neutralization titer with indicated antiserum ^a | | | | |
|---------------------|--|--------|-----------|----------|----------|
| | SMON-S | SMON-W | Baylor-12 | Baylor-9 | Baylor-6 |
| SMON-W | 10,000 | 5,000 | 1,780 | 110 | 220 |
| Baylor-12 | 10,000 | 5,000 | 1,780 | 110 | 200 |
| Baylor-1 | 6,400 | 2,800 | 710 | 90 | 1,600 |
| Baylor-9 | 3,200 | 2,200 | 560 | 110 | 1,600 |
| Baylor-6 | <25 | <25 | <25 | 110 | 2,500 |
| Baylor-24 | <25 | <25 | <25 | 110 | 2,500 |

^a Expressed as the 50% endpoint of serum dilution neutralizing 100 50% tissue culture infective doses of virus.

tralization titers (Table 3). IMV can be classified antigenically into three types; strains SMON-W and Baylor-12 are type 1, strains Baylor-6 and -24 are type 2, and strains Baylor-1 and -9 are an intermediate type with overlapping activity with types 1 and 2.

The results of antigenic analysis of IMV isolates from the United States from 1980 through 1983 indicated that these viruses can be classified antigenically into three types: type 1, type 2, and an intermediate type. However, all IMV isolates from Japan between 1969 and 1973 were identified as type 1 virus. It is apparent that more studies are required to determine the relationships among antigenic types, geographical distribution, epidemiological patterns, and disease spectrum.

It is practical to point out that intermediate type immune serum seems best for identifying a member of the IMV group. Type 1 antisera failed to react with type 2 virus, and

TABLE 3. Ratios of heterologous to homologous antiserum titers for IMV isolated in the United States

| Virus type | Virus strain tested | Ratio of heterologous to homologous titer with indicated antiserum ^a | | | | |
|--------------|---------------------|---|--------|-----------|----------|----------|
| | | SMON-S | SMON-W | Baylor-12 | Baylor-9 | Baylor-6 |
| 1 | SMON-W | 1.00 | 1.00 | 1.00 | 1.00 | 0.09 |
| | Baylor-12 | 1.00 | 1.00 | 1.00 | 1.00 | 0.08 |
| Intermediate | Baylor-1 | 0.64 | 0.56 | 0.40 | 0.82 | 0.64 |
| | Baylor-9 | 0.32 | 0.44 | 0.31 | 1.00 | 0.64 |
| 2 | Baylor-6 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |
| | Baylor-24 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |

^a Ranges of ratios were as follows: homotypic, 0.76 to 1.00; intermediate, 0.26 to 0.75; heterotypic, 0.00 to 0.25.

type 2 antisera would have to possess a high titer to neutralize type 1 virus. However, type 1 and type 2 sera seem valuable for determining type specificity.

LITERATURE CITED

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