Short Communication

SEROTYPING OF HUMAN ROTAVIRUS BY ENZYME IMMUNO-ASSAY WITH MONOCLONAL ANTIBODIES

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SUMMARY: Two series of enzyme immunoassays with monoclonal antibodies produced in two laboratories (A and B) were compared in use for serotyping of human rotavirus in stool samples collected in Japan between 1988 and 1991 from patients with gastroenteritis. Of 358 samples, 222 were determined to be the same serotype, while 61 samples could not be serotyped by either assay. A hundred and 92 samples were not serotyped by the A and B antibodies, respectively. We believe that the A and B monoclonal antibodies are useful clinically for serotyping rotaviruses at the present time.
Rotaviruses cause acute gastroenteritis in children. At least nine serotypes of group A human rotaviruses (HRVs), serotypes 1, 2, 3, 4, 6, 8, 9, 10 and 12, have been described on the basis of cross-neutralization studies with hyperimmune sera containing the neutralizing antibodies to the outer capsid polypeptides of VP7 (1-5). Serotypes 1, 2, 3 and 4 are most frequently detected in stool samples of rotavirus gastroenteritis (6,7).

Enzyme immunoassay with monoclonal antibodies (EIA-MAb) has been developed to determine serotypes directly from stool samples, particularly for epidemiological survey of circulating HRVs. Two series of EIA-MAb, A and B, have been prepared in Japan (4,8,9). In this study, we compared series A with B in serotyping rotaviruses in stool samples.

Three hundred and fifty-eight stool samples were collected in winter between the years 1988 and 1991 in Japan. The stool samples were diluted to about 10-20% suspensions with phosphate buffered saline (PBS). The samples were previously proved to be rotavirus positive by the latex agglutination test and polyacrylamide gel electrophoresis of genomic RNA. Any sample which contained two electropherotypes was not included in the present study.

Enzyme immunoassay with the A series of MAb

Serotypes 1-, 2-, 3- and 4-specific MAb (KU-4, S2-2G10, YO-1E2, and ST-2G7, respectively) and serotypes 1-4 common MAb (YO-156) were used. Wells of polyvinyl microtiter plates were coated with MAb diluted 1:10,000 in 10 mM PBS for a day at 4 C. They were then washed with PBST (PBS containing 0.05% Tween 20), then incubated with 1% bovine serum albumin in PBST for a day at 4 C before the final wash. A mixture of a 10% stool suspension (37.5 µl) and 10% skim milk (12.5 µl) was allowed to react overnight at 4 C in the wells. After washing, 1:10,000 diluted hyperimmune rabbit serum against the Wa strain was added and the plates were incubated at room temperature for 2 hr. After washing, 50 µl of peroxidase-conjugated goat anti-rabbit immunoglobulin G was added. The plates were incubated for one hour at 37 C and washed. The reaction with the enzyme substrates, o-phenylenediamine and hydrogen peroxide, was allowed to develop for 30 min at room temperature before the color was stabilized by the addition of 25 µl of 3 N sulfuric acid. The optical density (OD) was measured at 492 nm with a micro-ELISA reader.
Enzyme immunoassay with the B series of MAb

Serotypes 1-, 2-, 3-, and 4-specific MAb (BH49, BW36, BC5, and BE18, respectively) and serotypes 1-4 common MAb (BB4) were biotinylated before use. Unbiotinylated serotype common MAb (AH6) was used as the solid-phase (capture) antibody.

The wells of microtiter plates were each coated with 50 µl of a 1:100 diluted serotype-common MAb in carbonate-bicarbonate buffer (pH 9.6). After overnight incubation at 4 C, the plates were washed three times with PBST, and then 50 µl of SMT-PBST (PBST containing 2.5% skim milk powder) was added, followed by addition of 20 µl of a stool suspension (six wells for each sample). After 90-min incubation at room temperature or overnight incubation at 4 C, the plates were washed three times with PBST and 50 µl of 1:100 diluted biotinylated serotype-specific MAb was added to each well. After 90-min incubation at room temperature, the plates were washed again, and 50 µl of 1:1500 diluted peroxidase-conjugated streptavidin was added to each well. After 20 min, the plates were washed again, the enzyme substrate was added, then the reaction stabilized with sulfuric acid, and finally OD measured as described above (10).

With the A series of antibodies, we succeeded in serotyping 257 of the 358 samples, compared with 266 with the B series (Table I). None of the antibodies worked for serotyping other 61 samples.

With the A series, 87, 88, 39 and 43 samples were found to belong to serotypes 1, 2, 3 and 4, respectively, compared with 85, 73, 53 and 55 with the B series. In 222 samples, the two tests indicated the same serotypes. In four cases, however, the serotypes did not match, i.e., one sample was serotype 2 in the A series and serotype 1 in the B series, and the other three samples were serotype 2 in the A series and serotype 3 in the B series.

Rotavirus is an important agent of gastroenteritis all over the world, especially in developing countries. No effective anti-rotavirus drugs are known, so there is urgent demand for a vaccine. Recent vaccine trials have indicated that an effective vaccine should contain such strains as antigenically similar to those circulating in the community (12).

Epidemiological studies with MAbs have already shown serotype 1 to be the dominant type while serotypes 2, 3 and 4 are less commonly observed alternative types (6,7). For the present study, however, we selected stool samples containing an approximately equal number of each of these serotypes. The B series of antibodies was found to be slightly more sensitive than the A series in detecting sero-
Table I. Human rotavirus serotyping by enzyme immunoassay with two series of monoclonal antibodies (A and B)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>N. D.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>70</td>
<td>3</td>
<td></td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td></td>
<td>38</td>
<td></td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>41</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>N. D.</td>
<td>11</td>
<td>3</td>
<td>12</td>
<td>14</td>
<td>61</td>
<td>101</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>73</td>
<td>53</td>
<td>55</td>
<td>92</td>
<td>358</td>
</tr>
</tbody>
</table>

Note: N.D. indicates “not typable by the series.”

types 3 and 4, while the A series slightly more sensitive in detecting serotype 2.

The reasons speculated are as follows: (i) Urasawa et al. (11) originally used a polyclonal antibody mixture against the KU strain (serotype 1), the S2 strain (serotype 2), the YO strain (serotype 3) and the Hochi strain (serotype 4) instead of the Wa strain (serotype 1) used in the present study as detector antibodies. Furthermore, the peroxidase conjugated anti-rabbit antibodies may have been different. Such differences may have influenced the detection rates of the A series. (ii) MAbs recognize epitopes on viral proteins. Viruses which have different epitopes against MAbs may escape the reactivity of the A or the B series (6,8,13).

We believe that both series are useful for clinical studies of rotavirus infection at the present time.

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