Enzyme Immunoassay for Subgrouping Human Rotaviruses Using Monoclonal Antibodies

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Antibodies.* Tohoku J. exp. Med., 1984, **144** (1), 105-106 — Rotavirus group-
specific (common in human rotaviruses) and subgroup 1-specific monoclonal anti-
bodies were established and an enzyme immunoassay was developed for subgroup-
ing human rotaviruses. ——— enzyme immunoassay; subgroups; rotavirus;
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Rotavirus isolates have been typed into subgroups by complement fixation (Zissis and Lambert 1978), enzyme immunoassay (EIA) (Yolken et al. 1978) and immune adherence hemagglutination (Kapikian et al. 1981) using monospecific sera obtained by either absorbed hyper-immune serum or gnotobiotic calf postinfection serum. Since these antisera are difficult to obtain, developing monoclonal antibodies would be advantageous for use in these immunoassay systems.

We have fused spleen cells from mice immunized with KUN strain of rotavirus (Kutsuzawa et al. 1982) with X63-Ag8.653 cells to establish hybridoma clones which produce monoclonal antibodies reactive to rotaviruses. Two clones producing monoclonal antibodies, 133 and 152, were finally selected for use in an EIA for subgrouping. Wells of a microtiter plate were precoated with rabbit antibody directed against Wa virus. Test specimens containing rotaviruses were added to the wells in duplicate and incubated at 37°C for 2 hr. After washing with PBS containing Tween 20, monoclonal antibodies 133 and 152 were added to each well and incubated at 37°C for 2 hr. After washing, anti-mouse Ig conjugated with alkaline phosphatase was added and incubated at 37°C for 3 hr. After the final wash, p-nitrophenylphosphate was added and the color was measured at 405 nm. The positive result was defined as the extinction value 3 standard deviations greater than the mean value of the negative controls.

We selected 20 human rotavirus strains and compared their subgroup specificities with antigenic titers measured by our EIA (Table 1). The subgroup specificity of these strains was determined by RNA electrophoresis. All the rotavirus strains reacted to the monoclonal antibody 152. Furthermore, all 152 rotavirus strains detected by a conventional EIA could be detected by the antibody 152. However, only subgroup 1 rotaviruses, KUN and 83A001, reacted with a high titer to the monoclonal antibody 133. Together with the

TABLE 1. *Antigenic titers of 20 human rotavirus strains measured by monoclonal antibodies 133 and 152*

Virus	Subgroup*	Antigenic titer†	
		133	152
KUN (culture fluid)	1	4,096	2,048
Wa (culture fluid)	2	< 8	256
MO (culture fluid)	2	< 8	1,024
82A003 (5% stool suspension)	2	< 10	10,240
83A001 (5% stool suspension)	1	10,240	2,560
83A017 (5% stool suspension)	2	< 10	1,280
83A038 (5% stool suspension)	2	< 10	640
83A048 (1% stool suspension)	2	< 10	2,560
83A051 (5% stool suspension)	2	< 10	256
83A053 (5% stool suspension)	2	< 10	10,240
83A054 (5% stool suspension)	2	< 10	2,560
83A058 (5% stool suspension)	2	< 10	640
83A069 (5% stool suspension)	2	< 10	1,280
83A070 (5% stool suspension)	2	< 10	2,560
83A096 (5% stool suspension)	2	< 10	1,280
83A111 (5% stool suspension)	2	< 10	160
83A119 (5% stool suspension)	2	< 10	10,240
83A125 (5% stool suspension)	2	< 10	320
83A126 (5% stool suspension)	2	< 10	1,280
83A136 (5% stool suspension)	2	< 10	2,560

* Subgroup was determined by RNA electrophoresis.

† Reciprocals of the highest dilution giving positive EIA reading.

fact that these monoclonal antibodies reacted to the inner capsid particles (1.38 g/ml in CsCl) of the rotavirus which were known to possess rotavirus group and subgroup antigens, we therefore concluded that the monoclonal antibody 152 was a antibody directed to the group antigen common to all human rotaviruses and that the monoclonal antibody 133 was specific to subgroup 1 rotaviruses. By using these two monoclonal antibodies, human rotavirus field isolates will easily be typed into subgroup 1 (reactive to both 152 and 133) or subgroup 2 (reactive to 152 but not to 133).

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