JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1982, p. 727–730 Vol. 16, No. 4
0095-1137/82/100727-04$02.00/0
Copyright © 1982, American Society for Microbiology

# Isolation of Human Rotavirus Subgroups 1 and 2 in Cell Culture

TOYOKO KUTSUZAWA,[1] TASUKE KONNO,[1]* HIROSHI SUZUKI,[1] ALBERT Z. KAPIKIAN,[2] TAKUSABURO EBINA,[1] AND NAKAO ISHIDA[1]

*Department of Pediatrics and Department of Bacteriology, Tohoku University School of Medicine, Sendai 980, Japan,[1] and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205[2]*

Received 1 April 1982/Accepted 28 June 1982

One strain of human rotavirus subgroup 1 (KUN) and one strain of subgroup 2 (MO) were isolated with the MA104 cell line, a fetal rhesus monkey kidney cell line. Their subgroup specificities and RNA patterns were identical to those of rotaviruses present in stools before cultivation. Distinct cytopathic effects consisting of obscure cell boundaries, cell fusion, cell rounding, cell detachment, and lytic foci were recognized at passage 3 of MO and passage 6 of KUN. No differences in cytopathic changes were found between the two isolates.

Human rotavirus has been established as a major cause of acute gastroenteritis in infants and young children (4, 5). However, detailed virological and serological studies have been hampered by difficulties in propagating human rotavirus in cell culture systems (2). In 1980, the Wa strain, human rotavirus type 2, was adapted to grow efficiently in primary African green monkey kidney cell cultures after 11 passages in newborn gnotobiotic piglets (11). Recently, however, successful in vitro cultivation systems for human rotavirus, without any passages in animals, were reported by Sato et al. (8) and Urasawa et al. (9), and it was suggested that most of the human rotavirus strains detected in stools could be cultivated in cell cultures. In view of such successful cultivation, we aimed to isolate human rotaviruses which had been characterized as either subgroup 1 or subgroup 2 by immune adherence hemagglutination or enzyme-linked immunosorbent assay (3).

The MA104 cell line, an established cell line derived from the kidneys of embryonic rhesus monkeys, supplied by S. Matsuno, National Institutes of Health of Japan, were grown in Eagle minimum essential medium (MEM) containing 10% fetal calf serum and antibiotics (100 U of penicillin and 100 μg of streptomycin per ml). MA104 cells at passage 109 or more were grown in culture tubes (10 by 110 mm) for 2 to 3 days and used when confluent. All cultures were incubated at 37°C.

Two stool specimens, 80SR004 and 80SP001, containing a number of rotavirus particles detected by electron microscopy, were selected for this study. For analysis of the viral RNA patterns, fecal rotaviruses were purified by the method of Espejo et al. (1) and were then deproteinized with phenol. The pelleted RNA was dissolved in Laemmli sample buffer (7) and analyzed by polyacrylamide gel electrophoresis (6). The rotavirus of specimen 80SR004 was observed to have an RNA pattern with slow-moving segments 10 and 11 and was designated type S. The virus of specimen 80SP001 exhibited an RNA pattern with fast-moving segments 10 and 11 and was designated type L. Enzyme-linked immunosorbent assay or immune adherence hemagglutination subgroup specificities of the viruses were examined by the method described previously (3). The rotavirus with an RNA pattern of type S was subgrouped type 1, and that with a type L pattern was subgrouped type 2.

A 10% suspension in MEM of the stool specimens (80SR004 and 80SP001) was centrifuged at 10,000 rpm, and the clear supernatant fluids were mixed with an equal amount of 20 μg of trypsin per ml for 20 min. The mixture, containing rotavirus particles detected by electron microscopy, was diluted 1:20 with MEM before inoculation. The morphology of the rotaviruses changed very little after trypsin treatment, as shown by electron microscopy with negative staining. Before virus inoculation, confluent MA104 cell monolayers were washed three times with MEM without serum. Each tube, except for that containing uninfected controls, was inoculated with 0.2 ml of the diluted mixture. After incubation for 60 min at 37°C, the cultures were washed with MEM, fed with maintenance medium (MEM containing 0.5 μg of trypsin per ml), and then incubated in a roller drum at 37°C. The media were changed every

other day and checked by electron microscopy for the presence of virus particles. Eleven days after inoculation at the primary passage, the culture tubes were frozen, thawed, and then centrifuged. The supernatant fluid of the culture lysates were treated with trypsin (10 μg/ml) for 20 min at 37°C and then diluted 1:20 with MEM. The diluted culture lysates were inoculated onto fresh confluent monolayer cells for passage 2. At passage 2, the cell cultures were harvested 6 days after inoculation, and, after trypsin treatment, the culture fluids were inoculated into fresh MA104 cell cultures. At subsequent passages, the culture fluids for the next passage were obtained 2 or 3 days after inoculation and treated with trypsin as described above.

Cytopathic effects (CPE) were observed in the primary passage culture on day 10 in one of four culture tubes inoculated with stool specimen 80SR004, and on day 8 in two of four tubes and day 9 in three of four tubes, inoculated with specimen 80SP001. The cultivated rotavirus derived from 80SR004 was designated KUN, and that from 80SP001 was designated MO. At passage 2, CPE were noted on day 4 in one, day 5 in two, and day 6 in three of four culture tubes inoculated with KUN, and on day 4 in two, day 5 in three, and day 6 in four of four tubes inoculated with MO. However, distinctly recognizable CPE (Fig. 1) were not observed until the passage 6 of KUN and passage 3 of MO. Thereafter, CPE were recognized at 48 to 72 h after infection. CPE produced by these two rotavirus strains consisted of obscure cell borders, cell fusion, cell rounding, cell piling up, cell detachment from the surface of tubes, and lytic foci. Virus particles in the culture media were detected at passage 2 and subsequent passages (Fig. 2). The virus titer of the culture fluids was $10^{7}$ 50% tissue culture infective dose per ml of KUN at passage 8 and $10^{7.5}$ 50% tissue culture infective dose per ml of MO at passage 7.

To analyze RNA patterns of the tissue culture-adapted rotavirus strains, strain KUN at passage 6 and strain MO at passage 5 were grown in MA104 cell monolayers in roller bottles. Virus purification and electrophoresis of the viral RNA were carried out as described above.

The electrophoretic RNA patterns of tissue culture isolates KUN and MO were identical to those of their respective original rotaviruses present in stools (Fig. 3). Isolate KUN was type S, and MO was type L in regard to segments 10 and 11.

Subgroup specificity of the isolates grown in cell cultures was examined by immune adherence hemagglutination. KUN at passage 6 was found to belong to subgroup 1 (1:4 + <1:2 in antigen titer versus subgroup 1 + 2 infection serum, respectively), and MO at passage 5 was found to belong to subgroup 2 (<1:2 + 1:32 in antigen titer versus subgroup 1 + 2 infection serum, respectively), identical to the subgroup antigenicity of the respective original rotaviruses present in stools.

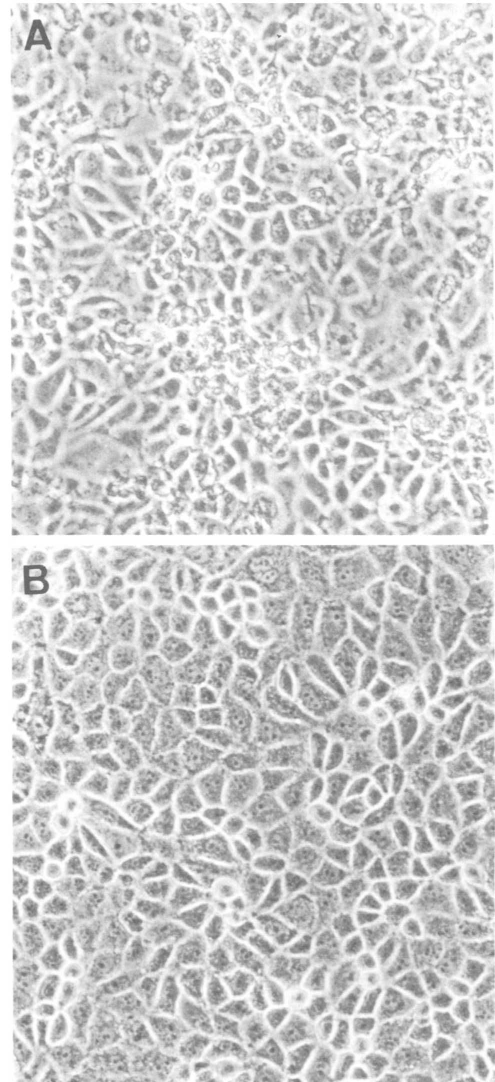


FIG. 1. CPE of strain MO in MA104 cells 74 h after infection (A) and in the uninfected control (B). The photographs were taken on a phase-contrast microscope after the culture medium had been changed.

Our results confirmed the in vitro cultivation methods of human rotaviruses which had been established by Sato et al. (8) and Urasawa et al. (9) in 1981. In this experiment, however, the cultures were incubated longer than those reported by other authors at passages 1 and 2 until CPE were demonstrated. Strain KUN, subgroup 1, was more difficult to grow in tissue culture as

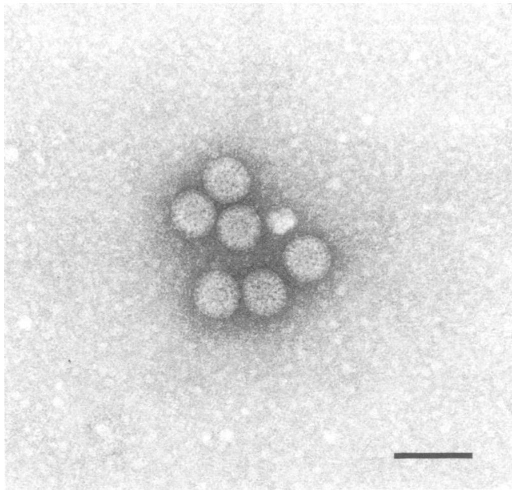

FIG. 2. Rotavirus particles of strain KUN in culture medium, negatively stained with uranyl acetate; ×100,000. Bar, 100 nm.

compared with tissue culture-adapted strain MO. Although it still remains to be proven, this difference may be related to the antigenic difference of human rotaviruses.

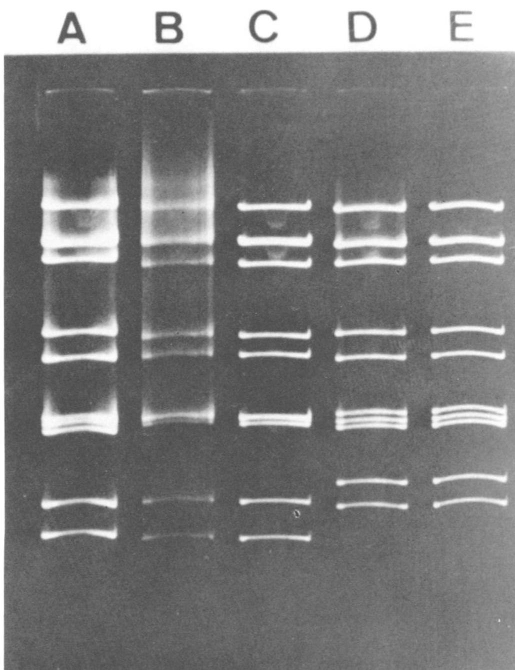


FIG. 3. Comparison of electrophoretic RNA patterns of human rotavirus stains KUN and MO and their virus origins purified from stools. A, Wa strain; B, original MO, C, cultivated MO (passage 5); D, original KUN; E, cultivated KUN (passage 6).

The culture-adapted strains KUN and MO were identified by immune adherence hemagglutination as subgroup 1 and 2, respectively, belonging to the same subgroups as the rotaviruses present in the stools (80SR004 and 80SP001, respectively). The RNA patterns of strains KUN and MO were also identical to those of the original stool rotaviruses. Thus, neither KUN or MO grown in MA104 cells appeared to be a contaminant of the tissue culture-adapted human rotavirus Wa strain whose RNA pattern was shown to be different from that of the KUN and MO strains. In addition, since no animal rotaviruses have ever been used for study in our laboratory, it is unlikely that the cultivatable rotaviruses KUN and MO were of animal origin.

The cultivation of two human rotaviruses, KUN and MO, with different antigenic properties in subgroup specificity, might facilitate a detailed study of human rotaviruses.

## ADDENDUM

We are grateful to R. G. Wyatt for determining the neutralization specificity of the cultivatable KUN and MO strains. The results revealed that KUN and MO represent distinct serotypes, DS-1 and M, respectively (10).

## LITERATURE CITED

1. **Espejo, R. T., L. F. Advendaño, O. Muñoz, P. Romero, J. G. Eternod, S. Lopez, and J. Moncaya.** 1980. Comparison of human rotaviruses isolated in Mexico City and in Santiago, Chile by electrophoretic migration of their double-stranded ribonucleic acid genone segments. Infect. Immun. **30**:342–348.
2. **Homes, I. H.** 1979. Viral gastroenteritis. Prog. Med. Virol. **25**:1–36.
3. **Kapikian, A. Z. W. L. Cline, H. B. Greenberg, R. G. Wyatt, A. R. Kalica, C. E. Banks, H. D. James, Jr., J. Flores, and R. M. Chanock.** 1981. Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): evidence for distinctness of IAHA and neutralization antigens. Infect. Immun. **33**:415–425.
4. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, S. L. Cline, R. H. Parrott, R. M. Chanock, J. O. Arrobio, C. D. Brandt, W. Rodriguez, A. R. Kalica, and D. H. Van Kirk.** 1976. Recent advances in the aetiology of viral gastroenteritis. Ciba Found. Symp. **42**:273–295.
5. **Konno, T., H. Suzuki, A. Imai, and N. Ishida.** 1977. Reovirus-like agent in acute epidemic gastroenteritis in Japanese infants: fecal shedding and serologic response. J. Infect. Dis. **135**:259–266.
6. **Kutsuzawa, T., T. Konno, H. Suzuki, T. Ebina, and N. Ishida.** 1982. Two distinct electrophoretic migration patterns of RNA segments of human rotavirus prevalent in Japan in relation to their serotypes. Microbiol. Immunol. **26**:271–273.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage $T_4$. Nature (London) **227**:680–685.
8. **Sato, K., Y. Inaba, T. Shinozuka, R. Fujii, and M. Matsumoto.** 1981. Isolation of human rotavirus in cell cultures. Arch. Virol. **69**:155–160.
9. **Urasawa, T., S. Urasawa, and K. Taniguchi.** 1981. Sequential passages of human rotavirus in MA-104 cells. Microbiol. Immunol. **25**:1025–1035.

10. **Wyatt, R. G., H. B. Greenberg, W. D. James, A. L. Pittman, A. R. Kalica, J. Flores, R. M. Chanock, and A. Z. Kapikian.** 1982. Definition of human rotavirus serotypes by plaque reduction assay. Infect. Immun. **37**:110–115.

11. **Wyatt, R. G., S. D. James, E. H. Bohl, K. W. Theil, L. F. Saif, A. R. Kalica, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1980. Human rotavirus type 2: cultivation *in vitro*. Science **207**:189–191.