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Preparation of a Human Rotavirus Reassortant with Dual Serotype Specificity, VP3 of Serotype 4 and VP7 of Serotype 3

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ABSTRACT **The reassortment of viral genome segments has been reported to occur during co-infection of cultured cells with two different rotaviruses. Further, epidemiologic findings suggesting that genetic reassortment of viral RNAs may account for an antigenic shift in rotavirus in nature have also been accumulated.**

In the present study a reassortant virus, C148, was selected from fifty-one progeny virus clones obtained under an antibody pressure from a mixed infection of MA104 cells with human rotavirus serotype 3 (YO) and serotype 4 (Hochi) strains. Antigenic characterization and genotype analysis by polyacrylamide gel electrophoresis concluded that C148 virus possessed a mosaic antigenicity defined by two serotype-specific viral proteins (VP), i. e. the serotype 4-specific VP3 and the serotype 3-specific VP7. While the reassortant C148 was judged to belong to serotype 3 on the basis of its preferential neutralizability by serotype 3 antiserum, the antiserum prepared against C148 equally neutralized both serotype 3 and 4 viruses.

These results seem to further support the possible emergence of a genetic reassortant in nature between human rotaviruses belonging to different serotypes.

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Key Words: Rotavirus, Genetic reassortment, Serotype, Viral protein

1. Introduction

Rotavirus particles contain a genome of 11 segments of double-stranded RNA which can be separated into distinct bands by gel electrophoresis, and the RNA gel electrophoretic pattern (electropherotype) has been used for the characterization and identification of rotavirus strains. Neutralization antigen of group A rotavirus is known to reside on the two outer capsid proteins, VP3 and VP7[1-5]. Cross neutralization tests have identified at least four different serotypes in group A human rotavirus[6-8] and recently fifth serotype has been proposed[9]. The serotype specificity was shown to be defined mainly by a glycoprotein VP7 which is encoded by RNA segment 8 or 9[10-13], whereas recently VP3 encoded by RNA segment 4 has also been shown to have an independent serotype specificity[4,5,14].

Previously we prepared in-vitro a reassortant of human rotavirus having a mosaic antigenicity defined by two serotype-specific antigens, namely the serotype 1-specific VP3 anigen and the serotype 2- specific VP7 antigen. The antigenic and genetic analysis of the reassortant was described[14]. Regarding its immunogenicity it was worthy of note that the antiserum raised against the reassortant equally neutralized both serotype 1 and serotype 2 strains. In the present study an antigenically mosaic reassortant strain was prepared by coinfection in-vitro with serotype 3 and serotype 4 human rotaviruses and was examined for its immunogenicity.

2. Materials and Methods

Screening of antigenic mosaic strain was performed in a manner similar to that described previously[14]. MA104 cell monolayers formed in a plastic 24-well microtitration plate (Falcon) were mixedly infected with the YO (serotype 3-subgroup II) and Hochi (serotype 4-subgroup II) strains pretreated with acetylated trypsin, at a multiplicity of infection of 2 p. f. u. /cell. After 48 hr incubation the virus yield from the mixedly infected culture was again treated with acetylated trypsin (final concentration, 10 μg/ml), diluted 10- to 100-fold, and plated on CV-1 cell monolayers in 5 cm Petri dishes. Plaques were allowed to develop under a purified agar overlay mdium[15] containing acetylated trypsin (3 μg/ml) and a 1 : 100 dilution of anti- Hochi (serotype 4) rabbit serum which had been made specific by absorption with concentrated serotype 3 YO strain (the neutralizing titers of the antiserum after absorption were 1 : 4,096 and $<$1 : 8 for Hochi and YO strains, respectively). Eighty-seven plaques developed were picked up, and propagated once in MA104 cell culture. Their reactivity against serotype 3 and 4 rotavirus antisera were examined in a fluorescent focus reduction neutralization test[2] using 1 : 2,000 diluted anti-YO and anti-Hochi rabbit sera (the neutralizing titers of both antisera against the homologous strains were 1 : 25,600). Fifty-one plaque progenies which were neutralized only with the YO antiserum were then screened for their reactivity to a VP3-directed monoclonal antibody ST-1F2[16], since the antibody ST-1F2 could distinguish between the VP3 antigens of Hochi and YO strains (the neutralizing titers of ST-1F2 were 1 : $\geqq$51,200 for Hochi stain and 1 : 1,600 for YO strain).

3. Results and Discussions

The screening test with the antibody ST-1F2 showed that only one plaque progeny (no. 148) of the 51 examined was significantly neutralized at a 1 : 10,000 dilution of the antibody.

Then the plaque progeny no. 148 was cloned by three plaque-to-plaque passages. The antigenic characters of a reassortant virus clone C148 thus obtained together with the parental strains are shown in Table 1. Virus clone C148 was neutralized by anti-YO rabbit serum as efficiently as the paental YO strain, while the neutralizing activity of anti-Hochi antiserum against the virus clone as well as YO strain was significantly low as compared with its activity against the parental Hochi strain, possibly indicating that the major serotype-specific protein VP7 of the clone C148 was derived from the YO parent. In contrast a similar reactivity of this virus clone and Hochi strain against monoclonal antibody ST-1F2 reacting to VP3 protein suggested that VP3 of this clone was derived from the Hochi parent. The YO-origin of VP7 of the clone C148 was also confirmed by its reactivity with monoclonal antibody YO-1E2 which was found to bind specifically to serotype 3-specific antigen on VP7 protein[17].

Table 1 *Antigenic characterization of reassortant C148*

Virus strain	Polyclonal antibodies		Monoclonal antibodies	
	Anti-YO (serotype 3) rabbit serum	Anti-Hochi (serotype 4) rabbit serum	ST-1F2	YO-1E2
C148	12800*	3200	$\geqq$51200	$\geqq$25600
YO	25600	1000	1600	$\geqq$25600
Hochi	500	25600	$\geqq$51200	$<$ 100

* neutralizing titer in a fluorescent focus neutralization test

The RNA gel electrophoretic patterns of the parental viruses and the reassortant virus C148 are shown in Fig. 1. The RNA segments 1, 2, 3, 4, 5, 6 and 10 of Hochi strain were distinguishable in polyacrylamide

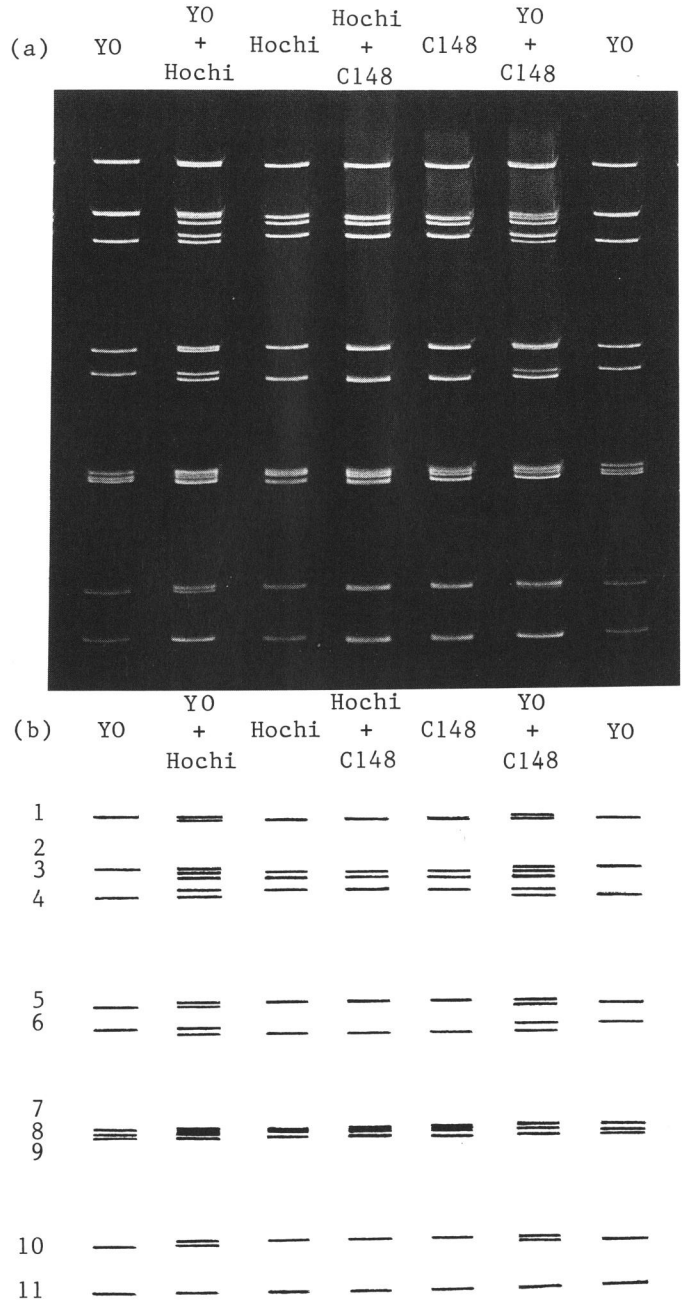

Fig. 1 Genotype analysis of reassortant C148 by PAGE.
(a) Comparison of the genome RNAs from reassortant C148 and parental YO and Hochi strains by co-electrophoresis.
(b) Schematic drawing of the same PAGE profiles.
Although Hochi origin of RNA segments 1, 5 and 10 of reassortant C148 is not clearly shown in the photograph (a), the direct analysis of this pattern on a UV transilluminator permitted this determination (b).

gel electrophoresis (PAGE) from the corresponding RNA segments of YO strain (lst to 3rd lanes from left). Co-electrophoretic comparison of the RNA from the reassortant virus with those of the parental viruses revealed the parental origin of most RNA segments of the reassortant virus (3rd to 7th lanes from left) : the reassortant contained RNA segments 1, 2, 3, 4, 5, 6, and 10 from the Hochi parent, while the origin of the other segments 7, 8, 9 and 11 remained undetermined. Recently RNA segment 4 is considered to code for VP3 protein of group A rotavirus[1,4,5,13,18]. The fact that RNA segments 1 through 6 including 4 of the reassortant are of Hochi origin indicates that the reassortant has VP3 of the Hochi parent (i. e. serotype 4), confirming the result of its antigenic characterization mentioned above. In contrast, VP7 of group A rotavirus is known to be coded for by either RNA segment 8 or 9 depending on the strain. Although the parental origin of RNA segment encoding VP7 of the reassortant could not be determined by the genotype analysis shown here, the result of antigenic analysis clearly indicated the YO origin of VP7 protein.

It has been reported that the immunization with a reassortant virus having dual serotype specificity induces antibodies against the two different serotypes[4,14]. To ascertain this point hyperimmune antiserum was prepared in weanling rabbits by two intravenous injections of the purified reassortant C148 as described previously[14]. Neutralizing activity of he antiserum against the reassortant C148 and two parental strains is shown in Table 2. The antiserum efficiently neutralized the two parental strains in addition to the reassortant itself, again confirming the mosaic antigenicity of this reassortant in terms of serotype specificity.

Table 2 *Neutalization of parental rotavirus strains, serotype 3 YO and serotype 4 Hochi by antiserum against reassortant virus, C148*

Virus	Neutralization titer of antiserum against C148
YO	4096
Hochi	4096
C148	8192

Previously, Hoshino *et al.* reported a field isolate of rotavirus, M37, which had VP3 protein of serotype 4 virus and VP7 protein of serotype 1 virus[4,19]. In addition, an in-vitro reassorted antigenic mosaic virus having serotype 1-specific VP3 and serotype 2-specific VP7 was also reported by the present authors[14]. These results suggest that genetic reassortment could actually occur, though not frequently, between rotaviruses of different serotypes, suggesting the possible emergence in nature of rotavirus strains having various serotype combinations. From a practical point of view it is expected that these reassortants are successfully employed to determine the protein specificity of various monoclonal antibodies[17].

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二種の血清型特異性，すなわち血清型4のVP3と血清型3のVP7を有する組換え体ヒトロタウイルスの作製

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2種のロタウイルスによる培養細胞の同時感染に際して，ウイルス遺伝子の組換えが起ることが報告されている．さらに，ウイルス遺伝子の組換えが，自然界におけるロタウイルスの抗原性の大変異の原因となっていることを示唆する疫学的知見も蓄積されて来ている．

本研究では，ヒトロタウイルス血清型3のYO株と血清型4のHochi株によるMA104細胞混合感染から，抗体存在下で得られた51のウイルスクローンより，組換え体ウイルスC148を選択した．抗原性の調査およびポリアクリルアミドゲル電気泳動によるウイルス遺伝子の分析から，C148ウイルスは2種の血清型特異的ウイルス蛋白により決定されるモザイク抗原性，すなわち血清型4特異的VP3と血清型3特異的VP7を有することが結論された．この組換え体C148は，血清型3抗血清による選択的易中和性から血清型3ウイルスと判定されたが，C148抗血清は血清型3および4ウイルスを共に同程度に中和した。これらの結果は，異なる血清型に属するヒトロタウイルスの間で，遺伝子組換え体が自然界で出現する可能性を一層強く支持するものと考えられる．