Preferential selection of VP7 gene from a parent rotavirus strain (SA11) in sequential passages after mixed infection with SA11 and SA11-human rotavirus single-VP7 gene-substitution reassortants

Brief Report


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Summary. We studied the competitive growth among SA11-L2(G3) and its single-human VP7 gene-substitution reassortants SA11-L2/KU-R1(G1) and SA11-L2/DS1-R1(G2), which have the genetic background of SA11-L2, during sequential passages after mixed infection. When the same infectious units (m.o.i. of 5 p.f.u./cell) of SA11-L2 and a reassortant SA11-L2/KU-R1 were inoculated onto and passaged in MA104 cells, 88% of the virus clones isolated from the culture fluid at the 3rd passage belonged to G3, and all the clones from the 10th passage had G3 specificity. Even when SA11-L2/KU-R1 with titer 10 times higher than that of SA11-L2 was used in the coinfection, the predominance of clones with G3-VP7 was observed. Although G2 clones slightly surpassed G1 clones in number in the mixed culture of SA11-L2/KU-R1 and SA11-L2/DS1-R1, G3 clones predominated in the virus progeny from a mixed culture infected with the same titers of SA11-L2, SA11-L2/KU-R1, and SA11-L2/DS1-R1. However, no significant difference in viral growth was detected among SA11-L2 and the two reassortants.

Group A rotavirus is an important viral agent of infantile diarrheal illness in humans. The fact that the diarrheal disease is the major cause of mortality in infants and young children especially in developing countries promotes the studies for the development of a rotavirus vaccine. The rotavirus particle consists of outer capsid and inner capsid layers containing 11 segments of double-stranded RNA(dsRNA). Two outer capsid proteins, VP7 and VP4, possess neutralization epitopes and are associated with independent antigenic...
specificities, G serotype and P serotype specificity, respectively. G serotypes 1, 2, 3, and 4, and P serotypes P1A and P1B (or VP4 genotypes represented by strains Wa or DS1) are most frequently detected in epidemiological studies on human rotavirus (HRV) [7, 15].

Although a number of strategies for rotavirus vaccination have been elaborated [6], up to date most experiences have been accumulated with oral administration of live attenuated rotaviruses of bovine (RIT4237 and WC3) or simian (rhesus rotavirus strain MMU18006) origin. Furthermore, since such live vaccine strains could not induce satisfactory antibody responses against all the four major HRV G serotypes 1–4, multivalent vaccines composed of RRV with G3 specificity (or WC3 with G6 specificity) and its single-human VP7 gene-substitution reassortants with G1, G2, and G4 specificity have been developed [3, 11, 12]. Although safety and immunogenicity of the multivalent vaccines have been evaluated in several field trials [4, 8, 14], the presence of ‘interference’ among the vaccine component strains was suggested by the finding that the antibody response rates to the four HRV G serotypes in vaccinees were not uniform even after the same infectious units of vaccine components were orally administered [5, 14]. However, no detailed investigation has been made in vitro or in vivo on the possible ‘interference’ among the vaccine components.

Our present study was carried out to analyse this issue in vitro using simian rotavirus strain SA11-L2(G3) [17] and its reassortants with a single-human G1- or G2-VP7 gene as a model of RRV-based multivalent vaccines. Two single-VP7 gene-substitution reassortants, SA11-L2/KU-R1(G1) and SA11-L2/DS1-R1(G2) were obtained from mixed culture of SA11-L2 and a G1 human strain KU or SA11-L2 and a G2 human strain DS1, through repeated selections with anti-VP7 G3-specific neutralizing monoclonal antibody (N-MAb) (McN-10) and anti-HRV VP4 N-MAb (S3–2C or S3–5E) as described previously [9]. Except for the VP7 gene, the other 10 RNA segments of these reassortants were derived from SA11-L2.

The following three combinations of mixed infections of rotaviruses were performed: A, SA11-L2 x SA11-L2/KU-R1; B, SA11-L2/KU-R1 x SA11-L2/DS1-R1; C, SA11-L2 x SA11-L2/KU-R1 x SA11-L2/DS1-R1. Each virus was pretreated with 20 µg/ml of acetylated trypsin at 37 °C for 1 h and simultaneously inoculated onto MA-104 cells at a multiplicity of infection (m.o.i.) of 5 plaque-forming units/cell. Sequential virus passage was performed by inoculating mixed virus culture fluid onto MA-104 cells (at an approximate m.o.i. of 5 p.f.u./cell of viruses) and incubating cells for 2 days at each passage.

From culture fluid of the 3rd and the 10th passages of the mixed infections A, B, and C, more than 50 clones of each were randomly isolated by plaque-formation in CV-1 cells, and propagated in MA-104 cells. G serotype of each clone was determined by ELISA using G serotype 1, 2, and 3-specific monoclonal antibodies (MAbs) KU-6BG, S2-2G10, and McN-10, respectively, as described previously [16]. Table 1 indicates G serotype distribution of a total of 328 clones isolated from mixed cultures A, B, and C in which viruses with equal titers are infected.