Rearrangement of Genomic Segment 11 in Two Swine Rotavirus Strains

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SUMMARY

We have recently reported the isolation of two group A swine rotaviruses each lacking normal genomic RNA segment 11 and showing instead one extra segment that migrated abnormally on gel electrophoresis. Hybridization studies performed with segment-specific probes and with a purified abnormal RNA segment showed that the extra bands had sequence homology to normal segment 11. Analysis of protein profiles of normal and rearranged strains showed that the gene product of segment 11 had no apparent change in its relative electrophoretic migration, suggesting that the rearranged genes remained functional.

It is now widely accepted that the rotavirus genus is composed of groups of viruses which are not antigenically related, each one displaying a characteristic electropherotype. Recently, several group A rotaviruses with abnormal dsRNA migration profiles have been reported (Pedley et al., 1984; Besselaar et al., 1986; Pocock, 1987; Bellinzoni et al., 1987). Rotaviruses with atypical genomic RNA migration patterns have also been generated in tissue culture after serial passages at high m.o.i. (Hundley et al., 1985). We have recently reported the isolation of two different group A swine rotaviruses containing 11 dsRNA segments with an unusual distribution (Bellinzoni et al., 1987). The two strains lack the normal segment 11 but have a new band after electrophoresis. In one case, the abnormal band is positioned between segments 6 and 7 of the normal pattern (C60 strain) and in the other case the abnormal band is located just ahead of normal segment 9 (C117 strain).

Adaptation of the strains studied in this paper to grow in tissue culture was similar to that previously described (Bellinzoni et al., 1987). When first assayed, suspensions of faeces containing C60 and C117 strains produced a marked c.p.e. on MA-104 cultures, 24 h post-infection. Lysates of these infected monolayers were used as secondary inocula and no longer induced c.p.e. on MA-104 cells in spite of the presence of large amounts of intracellular viral antigen as revealed by an immunoperoxidase staining assay (IPA) (Gerna et al., 1984). It seems that when these virus strains are assayed in MA-104 stationary cultures, they failed to lyse infected cells thus preventing the spread of the virus to neighbouring cells. When cultured in roller bottles, however, both strains caused c.p.e. Since it was not possible to plaque purify the C60 and C117 viral strains, they were both cloned four times by the limiting dilution method.

Strain C117 was originally isolated from herds of pigs where, in some cases, it coexisted with viruses having the normal electropherotype. Moreover, another strain (Cc86), with a rearranged pattern similar to that of strain C117, was later obtained during the process of cloning viruses with normal patterns obtained from the same herd (CN86). Interestingly in this case, all but one of the genomic segments comigrated (see Fig. 1) These viruses were also cloned by the limiting dilution method. In contrast strain C60 was isolated from animals from which viruses with a normal pattern were not detected. A year later, viruses with a C60-like electropherotype were isolated from the same herd (C134 strain). The electrophoretic profile of the strains described
Fig. 1. Genome profiles of standard and rearranged strains. Genome segments from strains C60 (lane 1), OSV (lane 2), C134 (lane 3), CN86 (lane 4), Cc86 (lane 5) and C117 (lane 6) were separated in 10% polyacrylamide gels and visualized by silver staining (Herring et al., 1982). Lane 6 is a sample run on a different gel.

Fig. 2. Hybridization analysis of virus dsRNA. Genomic dsRNAs of strains C60 (lanes 3) and C117 (lanes 4) were separated by PAGE and stained with silver nitrate (a). Similar gels were electroblotted to Gene Screen nylon membranes and hybridized to 32P-labelled clones specific for bovine segments 10 and 11 (b) or to 32P-labelled X1 segment (c). Swine OSU (lanes 2) and bovine UK (lanes 1) strains were included for comparison. Hybridizations were carried out in 3 x SSC, 5 x Denhardt's solution, 50 µg/ml sonicated salmon sperm DNA and 0.1% SDS at 65 °C and washed three times in 0.1 x SSC and 0.1% SDS at the same temperature. Each panel corresponds to different gels run under the same conditions. Lanes of panels (b) and (c) correspond, in each case, to different tracks of the same gel.

above is shown in Fig. 1. It can be observed that C60, C134, C117 and Cc86 have no band at the position where segment 11 normally migrates (approx. 670 base pairs). There is instead, in each case, a new band located at an atypical position for a group A pattern, indicated as X1 (C60 and C134 strains) of approx. 1200 bp and X2 (C117 and Cc86) of approx. 900 bp in length.

In a first attempt to determine the origin of segments X1 and X2, Northern blots (Street et al., 1982) were hybridized to different segment-specific cDNA clones derived from a local bovine rotavirus strain (Crespi et al., 1986). It was found that X1 and X2 hybridized with only a full-length segment 11 cDNA probe, as shown in Fig. 2(b). A cDNA clone corresponding to a bovine segment 10 was also included in this experiment, hybridizing exclusively with segment 10 of all four samples. In influenza virus, genome rearrangement elicited mosaic structures involving more than one segment sequence (Fields & Winter, 1982). This appears not to be the case with the C60 and C117 rotaviruses, since hybridization with clones specific for segments 4 to 10 gave no cross-hybridization signals with segments X1 and X2 (data not shown). To investigate this point further, segment XI was electroeluted from a polyacrylamide gel (Maniatis et al., 1982) and, after partial alkaline hydrolysis and 32P 5' end-labelling (Arrand, 1985), used as a probe. Fig. 2(c) shows that this probe hybridized only to itself, to segment X2 and to genomic segment 11 of swine UK and OSU strains. This experiment suggests that none of the rearranged bands represent sequences corresponding to segments other than those of normal segment 11. The rearranged segments may contain very short regions from other RNA segments which might not
Fig. 3. Profile of virus-induced proteins in MA-104 cells. Monolayers were labelled for 60 min at 6 h post-infection with 100 μCi/ml of [35S]methionine (New England Nuclear; sp. act. 1100 Ci/mmol). When indicated, tunicamycin was used at 1μg/ml 60 min before labelling. Cells lysed in Laemmli’s sample buffer (Laemmli, 1970) were analysed on a 10% polyacrylamide gel. Lane 1, mock-infected cells; lane 2, mock-infected cells with tunicamycin; lane 3, OSU strain; lane 4, OSU strain with tunicamycin; lane 5, C60 strain; lane 6, CN86 strain; lane 7, Cc86 strain; lane 8, C117 strain. Proteins are named according to McCrae & Faulkner-Valle (1981).

be detected by whole segment hybridization probes or that they contain sequences not homologous to the rotavirus genome.

In order to study whether these rearrangements have any consequence at the protein level, we analyzed the virus coded proteins of strains C60, C117 and Cc86 by SDS-PAGE after [35S]methionine labelling of infected MA-104 cells. The three rearranged strains induced a polypeptide not present in mock-infected cells, which migrated in the position of the gene product of segment 11 (VP 11 27000 Mr) of the standard OSU strain (Fig. 3). Moreover, in spite of the difference in the RNA genomic pattern of the rearranged Cc86 and normal CN86 strains, the overall protein profiles were identical. The labelling of infected cells performed in the presence and absence of tunicamycin allowed us to localize precisely the position of VP11 in the gel, due to the change in mobility of unglycosylated VP10 (McCrae & Faulkner-Valle, 1981).

The results presented in this work indicate that the atypical group A swine rotaviruses described have genomic rearrangements derived from normal RNA segment 11 sequences, that migrate as larger dsRNA genomic segments. They did not appear to be mosaic structures of the remaining segments. Similar cases involving genomic rearrangement of segment 11 have been described in bovine (Pocock, 1987), rabbit (Thouless et al., 1986) and human rotaviruses isolated from immunodeficient children (Pedley et al., 1984). Orbivirus variants containing either RNA deletions or concatemeric RNAs have also been described (Eaton & Gould, 1987). It is worth mentioning that although both strains retained their rearranged genome after cloning, they were still virulent in piglets. Moreover, viruses with a C60-like electropherotype were detected in the
field after 2 years of C60 isolation. Analysis of the proteins induced in cells infected with strains C60, C117 and Cc86 indicated that VP11, the protein encoded by normal segment 11, was still present. A similar result has also been described in a bovine rearranged strain (Pocock, 1987). It is therefore likely that the rearrangements that led to segments X1 and X2 did not alter the protein coding region of genomic segment 11.

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