Cell Culture Adaptation of Astrovirus Involves a Deletion

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Astroviruses have been adapted to culture by serial blind passage in primary human embryo cells. All viruses thus adapted possess a 45-nucleotide deletion relative to fecal viruses or isolates made in CaCo-2 cells; this deletion may be responsible for the change in host cell range.

Human astroviruses (HAst) were identified in diarrheal stools by electron microscopy (7) and were named for the star-shaped motif visible on the surface of the particles (8). Astroviruses are now associated with gastroenteritis in both animals and humans (3). Human fecal astroviruses could only be grown in continuous cell cultures following six cycles of blind passage in primary human embryo kidney (HEK) cells. No cytopathic effects were seen until the sixth passage. Viruses so isolated could grow in a continuous cell line (LLC-MK2). However they could not replicate in other kid cells such as Vero cells (5). Cell culture-adapted prototype strains of the five serotypes of human astrovirus were isolated by this method (2); we refer to such prototype viruses as HAst-1p through HAst-5p. These isolates are host range mutants and have been used for most astrovirus experimentation. Recently, human fecal astroviruses were cultured directly in a continuous cell line (CaCo-2) derived from a human colonic carcinoma (13). This removed the requirement for blind-passage adaptation.

Sequence information is now available for two prototype human astroviruses adapted to growth in LLC-MK2 cells as described above: a serotype 1 (HAst-1p [9]) astrovirus and a serotype 2 (HAst-2p [1]) astrovirus; and also from a serotype 1 astrovirus isolated in CaCo-2 cells (HAst-1 A2/88 [11]). The virus genome consists of three open reading frames (ORFs). The two open reading frames at the 5' end (ORFs 1a and 1b) contained motifs indicative of nonstructural proteins and were linked by a ribosome frameshift such that the RNA-dependent RNA polymerase was encoded in ORF 1b and the viral protease and other motifs were located in ORF 1a (1, 6, 11). The remaining ORF at the 3' end (ORF 2) encodes the structural proteins and is expressed via a subgenomic RNA (10, 12).

Comparison of the complete genomic sequence of a H-Ast1 virus isolated in CaCo-2 cells (A2/88 Newcastle) with those of the two cell-adapted isolates showed close similarity in the 1,430-amino-acid nonstructural protein. A total of 15 amino acid substitutions occurred between the proteins of the two serotype 1 viruses (one prototype and one isolated in CaCo-2 cells), 12 of which maintained amino acid type (polar, etc). The most striking feature was a region in ORF 1a (commencing at amino acid 792 in the sequence of HAst-1 A2/88) in which A2/88, grown in CaCo-2 cells directly from diarrheal feces, possessed 15 amino acids (or 45 nucleotides) absent in the prototype viruses of both serotypes 1 and 2. We have suggested that this difference may result from the cell culture adaptation process (11), and we have now examined this process more closely by using a reverse transcription-PCR to amplify the deletion site from a number of different viruses. The sequences of HAst-1p and HAst-2p have been presented elsewhere (1, 9). We obtained the remaining three prototype strains of human astrovirus serotypes (HAst-3p through HAst-5p) isolated in Oxford (5). An additional isolate of a serotype 4 virus was made in CaCo-2 cells at Great Ormond St Hospital, London (HAst-4 GOS). We have used inocula derived from passages 4 and 10 in these cells. Two further astrovirus samples were obtained as diarrheal feces containing serotype 1 astroviruses. The first (HAst-1 A1/88) was from Newcastle upon Tyne, United Kingdom (13), whereas the other originated in Oxford, United Kingdom. Samples of those stools from which the prototype viruses were originally obtained (prior to 1971) were no longer available, but one such stool sample (that for serotype 1) had also been used for pathogenesis studies in volunteers. One of these had developed diarrhea and shed astrovirus in the feces (4). This material, removed by a single passage in the human gut from the progenitor of HAst-1p, was available and was used in this study. We have termed this volunteer's sample HAst-1V. Each sample was inoculated onto CaCo-2 cells, and RNA was isolated as described previously (13). Primers selected for the reverse transcription-PCR were 5'-TGAATTAATCCAGATTTGAT-3' for the reverse transcription primer and 5'-CCTGCCGAGAAAACGAG-3' for the positive primer. In each case, total cellular RNA was reverse transcribed and amplified by PCR using Taq DNA polymerase (Boehringer Mannheim). Forty cycles of amplification were performed as follows: 94°C, 30 s; 55°C, 30 s; and 72°C, 30 s. The reaction products were sized on 2% agarose- Trisborate-EDTA gels, and PCR products were sequenced directly.

The PCR products obtained from the sequenced viruses were as expected. In each case, PCR products derived from viruses isolated in CaCo-2 cells were larger than those of prototype strains by approximately 50 bases. All PCR products were sequenced, and the predicted translation products from these and from published sequences are aligned in Fig. 1. All products from the three new prototype viruses (serotypes 3, 4, and 5) had the same in-frame deletion of 45 bases as the two prototype viruses already sequenced (serotypes 1 and 2). Furthermore, this deletion was absent from HAst-1V derived from the progenitor of the serotype 1 prototype virus. Thus the presence or absence of these 15 amino acids cannot be accounted for by strain differences between the isolates, and the deletion is likely to be a product of the cell adaptation process. This is also supported by the results obtained from serotype 4 virus. Again only the prototype strain, adapted to growth in human embryo cells, showed this deletion. Type 4

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viruses, isolated and passaged up to 10 times in CaCo-2 cells, retained these 15 codons. Since astroviruses are unable to grow in LLC-MK2 cells without prior passage through HEK cells, and since CaCo-2 cell-grown virus which lacks this deletion also fails to grow in LLC-MK2 cells, it seems likely that growth in HEK cells has resulted in the loss of these amino acids and acquisition of the ability to grow in LLC-MK2 cells.

The deletion removes amino acids from a partially repetitious region leaving only a single copy of each of the repeated sequences EQQVK and KPQ (Fig. 1). This part of the nonstructural protein is of unknown function and is located towards the 3’ end of ORF 1a some 100 amino acids before the frameshifting sequence at the end of this ORF where some 90% of proteins are terminated. ORF 1a also encodes the viral protease and, closer to the deletion region, a nuclear targeting motif. Thus, the deletion may affect a protein transported to the nucleus; any such transport would suggest interaction with host cell machinery, and adaptive deletion may be required to improve the efficiency of this process.

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