Letters in Applied Microbiology 2005, **41**, 103–105 doi:10.1111/j.1472-765X.2005.01759.x

UNDER THE MICROSCOPE

Human astrovirus diagnosis and typing: current and future prospects

S. Guix, A. Bosch and R.M. Pintó
Enteric Virus Group, Department of Microbiology, University of Barcelona, Barcelona, Spain

2005/0391: received 13 April 2005 and accepted 19 April 2005

SUMMARY

Human astroviruses (HAstV) are important human pathogens causing gastroenteritis worldwide. The increased recognition of astroviruses as the cause of sporadic outbreaks of disease is due to the recent availability of improved diagnostic methods. During the last decade, most epidemiological surveys have chosen astrovirus-specific RT-PCR as screening methods. In addition to serotyping by molecular techniques, new typing methods are being developed that may also identify other viral properties related to virulence. The information provided by different typing assays is required for a better understanding of both the antigenic diversity and the molecular mechanisms of pathogenicity.

BACKGROUND ON HUMAN ASTROVIRUSES

Human astroviruses (HAstV) belong to the *Astroviridae* family, which is divided into two genera: *Mamastrovirus* (mammal astroviruses) and *Avastrovirus* (avian astroviruses). The virion (28–41 nm) is nonenveloped and contains a positive single-stranded polyadenylated RNA genome, which includes a 5′-untranslated region(UTR), three overlapping open reading frames (ORFs) and a 3′-UTR. ORF1a and ORF1b code for the nonstructural proteins, such as the serine protease and the RNA-dependent RNA polymerase, while ORF2 encodes the capsid precursor (Matsui and Greenberg 2001).

Surveillance using molecular diagnostic methods developed in the past two decades shows that HAstV are one of the most important causes of acute paediatric gastroenteritis, together with rotavirus and calicivirus (Glass *et al.* 1996; Walter and Mitchell 2003). Generally, the infection produces diarrhoea of short duration sometimes accompanied with fever and vomiting, although protracted diarrhoea has been associated with serotype 3 strains (Caballero *et al.* 2003). HAstV infections occur in all countries where a study has been carried out and affect mainly young children, and the elderly, with an average incidence of 2–9% in developed countries.

Correspondence to: Albert Bosch, Department of Microbiology, University of Barcelona, Avda Diagonal 645, 08028 Barcelona, Spain (e-mail: abosch@ub.edu).

Currently, there are eight serotypes of HAstV, type 1 being the most prevalent worldwide. Epidemiological observations suggest that HAstV infections do not induce heterotypic immunity, as an episode of HAstV diarrhoea is not associated with a reduced incidence of a subsequent episode (Naficy *et al.* 2000). The existence of homotypic immunity is still uncertain. Phylogenetic analysis consistently show that it is common to find multiple HAstV strains circulating in one region during a given period of time, and that there are also variations in the prevalent type with time, suggesting either a genetic shift or an introduction of new strains (Mustafa *et al.* 2000; Naficy *et al.* 2000; Walter *et al.* 2001; Guix *et al.* 2002; Jakab *et al.* 2003; De Grazia *et al.* 2004). Specifically, a replacement of the prevalent serotype 1 subtype over a period of time has been observed (Guix *et al.* 2002; Jakab *et al.* 2003; De Grazia *et al.* 2004), suggesting a lack of homotypic immunity. However, as serotype 1 is the most prevalent, and there have been no reports of children suffering subsequent episodes of diarrhoea caused by this type, some authors argue that HAstV infection may induce homotypic immunity (Naficy *et al.* 2000).

RECENTLY DEVELOPED DIAGNOSTIC AND TYPING TOOLS

Although several laboratories have developed alternative diagnostic methods to simultaneously identify multiple

Table 1 Most commonly used RT-PCRs for HAstV detection

Primers	Genomic region	Length (bp)	References
Common-type			
Mon340/Mon348	ORF1a (pro)	289	Belliot *et al.* 1997
A1/A2	ORF1a (C-terminal nsP1a)	192–237	Guix *et al.* 2002; Willcocks *et al.* 1994
Mon343/Mon344	ORF1b (RNA pol)	316	Belliot *et al.* 1997
Mon244/Mon245	ORF2 (N-terminal)	413	Noel *et al.* 1995
Mon269/Mon270	ORF2 (N-terminal)	449	Noel *et al.* 1995
prBEG/Mon2	ORF2 (C-terminal) + 3′-UTR	296–324	Saito *et al.* 1995
DM4/Mon2	ORF2 (C-terminal) + 3′-UTR	1200	Walter *et al.* 2001
Mon2/Mon69	3′-UTR	89	Mitchell *et al.* 1995
Type-specific			
AST-S1 to AST-S8, FOR, END	ORF2 (C-terminal)	118–599	Matsui *et al.* 1998; Sakamoto *et al.* 2000
PR6151, PR6257, DM12, JWT4, AST-S5, DM11, Mon2	ORF2 (C-terminal) + 3′-UTR	321–666	Saito *et al.* 1995; Mitchell *et al.* 1999; Walter *et al.* 2001

enteric pathogens such as astrovirus, norovirus, enterovirus and adenovirus by multiplex real-time PCR (Beuret 2004; Rohayem *et al.* 2004; Yan *et al.* 2003), in general, astrovirus-specific RT-PCR has been the screening method of choice to detect HAstV in stool samples in most large-scale epidemiological studies. While some authors have used highly sensitive primers targeted to conserved genomic regions coding for the nonstructural proteins and UTRs (Mitchell *et al.* 1995; Belliot *et al.* 1997; Guix *et al.* 2002), other authors prefer to use primers from the capsid coding region which can be less sensitive but provide type information (Noel *et al.* 1995; Saito *et al.* 1995; Walter *et al.* 2001) (Table 1). Based on the nucleotide variability of either the 5′-end or the 3′-end of ORF2, it is now possible to phylogenetically group the eight HAstV serotypes. Three of the most commonly used RT-PCR serotyping methods are based on this variability, either using common primers for all types plus a sequencing reaction (Noel *et al.* 1995), or using type-specific primers and determining the serotype according to the size of the amplified product (Matsui *et al.* 1998; Mitchell *et al.* 1999; Sakamoto *et al.* 2000; Walter *et al.* 2001).

In addition, according to genetic diversity of the ORF1a, HAstV strains are also classified into two clearly differentiated genogroups, designated genogroup A and genogroup B (Belliot *et al.* 1997). Although genogroup A includes serotypes 1–5 and 8, and genogroup B includes serotypes 6 and 7, there is not a complete correlation between sequence variability and serotype. Instead, variability within the hypervariable region (HVR) close to the C-terminus of nsP1a has been associated to different viral RNA replication and growth properties, as well as to different RNA loads in faeces from children with gastroenteritis, suggesting a relationship between certain genotypes and some viral properties related to its pathogenic phenotype (Guix *et al.* 2004, 2005). Variability within this region consists mainly of high rates of insertions and deletions that maintain the reading frame. This phenomenon has motivated the development of typing methods, which could trace these differences in virulence (S. Guix, S. Caballero, R. M. Pintó and A. Bosch in preparation). If affordable, typing of isolates using different systems would be advisable, not only because different genomic regions may provide medically relevant information, but also because it would also increase the chances to identify new variants of HAstV generated by recombination.

CONCLUSIONS

The importance of astroviruses as human pathogens has increased with the widespread use of molecular techniques in epidemiological studies. Knowledge of the molecular epidemiology of a virus is the key step in understanding its burden in human health, and availability of good detection and typing methods are crucial. The use of different typing methods that provide information regarding serotype and other pathogenic properties may be advisable to further characterize the HAstV strains that circulate in our communities. Not only it would help to analyse the extent of genetic diversity, but it would also be useful to detect the emergence of possible recombinant strains.

FUTURE DIRECTIONS

With the improvement of detection and typing methods, both the incidence and the extent of genetic variability of

HAstV have increased. In order to clarify whether the burden of HAstV infections is still being underestimated, we should keep using the newly developed tools in large-scale epidemiological studies. Additionally, more studies on genetic and antigenic diversity are required to elucidate the mechanisms of HAstV-induced immune response, prior to attempting the development of a vaccine. Thus, it still has to be ascertained whether HAstV infection generates heterotypic and/or homotypic protection. Finally, studies on HAstV genomic organization and molecular mechanisms of pathogenicity would also provide new insights into the understanding of the disease caused by HAstV and could help identify possible targets for antiviral drugs.

REFERENCES

Belliot, G., Laveran, H. and Monroe, S.S. (1997) Detection and genetic differentiation of human astroviruses: phylogenetic grouping varies by coding region. *Arch Virol* **142**, 1323–1334.

Beuret, C. (2004) Simultaneous detection of enteric viruses by multiplex real-time RT-PCR. *J Virol Methods* **115**, 1–8.

Caballero, S., Guix, S., El-Senousy, W.M., Calicó, I., Pintó, R.M. and Bosch, A. (2003) Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. *J Med Virol* **71**, 45–50.

De Grazia, S., Giammanco, G.M., Colomba, C., Cascio, A. and Arista, S. (2004) Molecular epidemiology of astrovirus infection in Italian children with gastroenteritis. *Clin Microbiol Infect* **10**, 1006–1035.

Glass, R.I., Noel, J., Mitchell, D., Herrmann, J.E., Blacklow, N.R., Pickering, L.K., Dennehy, P., Ruiz-Palacios, G. *et al.* (1996) The changing epidemiology of astrovirus-associated gastroenteritis: a review. *Arch Virol (Suppl)* **12**, 287–300.

Guix, S., Caballero, S., Villena, C., Bartolomé, R., Latorre, C., Rabella, N., Simó, M., Bosch, A. *et al.* (2002) Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol* **40**, 133–139.

Guix, S., Caballero, S., Bosch, A. and Pintó, R.M. (2004) C-terminal nsP1a protein of human astrovirus colocalizes with the endoplasmic reticulum and viral RNA. *J Virol* **78**, 13627–13636.

Guix, S., Caballero, S., Bosch, A. and Pintó, R.M. (2005) Human astrovirus C-terminal nsP1a protein is involved in RNA replication. *Virology* **333**, 124–131.

Jakab, F., Walter, J.E., Berke, T., Matson, D.O., Mitchell, D.K. and Szucs, G. (2003) Molecular characterization and sequence analysis of human astroviruses circulating in Hungary. *FEMS Immunol Med Microbiol* **39**, 97–102.

Matsui, M., Ushijima, H., Hachiya, M., Kakizawa, J., Wen, L., Oseto, M., Morooka, K. and Kurtz, J.B. (1998) Determination of serotypes of astroviruses by reverse transcription-polymerase chain reaction and homologies of the types by the sequencing of Japanese isolates. *Microbiol Immunol* **42**, 539–547.

Matsui, S.M. and Greenberg, H.B. (2001) Astroviruses. In *Fields Virology* ed. Fields, B.N., Knipe, D.M., Howley, P.M., Griffin, D.E., Martin, M.A., Lamb, R.A., Roizman, B. and Straus, S.E. pp. 875–893. Philadelphia, PA: Lippincott Williams & Wilkins.

Mitchell, D.K., Monroe, S.S., Jiang, X., Matson, D.O., Glass, R.I. and Pickering, L.K. (1995) Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase-polymerase chain reaction. *J Infect Dis* **172**, 1437–1444.

Mitchell, D.K., Matson, D.O., Jiang, X., Berke, T., Monroe, S.S., Carter, M.J., Willcocks, M.M. and Pickering, L.K. (1999) Molecular epidemiology of childhood astrovirus infection in child care centers. *J Infect Dis* **180**, 514–517.

Mustafa, H., Palombo, E.A. and Bishop, R.F. (2000) Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. *J Clin Microbiol* **38**, 1058–1062.

Naficy, A.B., Rao, M.R., Holmes, J.L., Abu-Elyazeed, R., Savarino, S.J., Wierzba, T.F., Frenck, R.W., Monroe, S.S. *et al.* (2000) Astrovirus diarrhea in Egyptian children. *J Infect Dis* **182**, 685–690.

Noel, J.S., Lee, T.W., Kurtz, J.B., Glass, R.I. and Monroe, S.S. (1995) Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J Clin Microbiol* **33**, 797–801.

Rohayem, J., Berger, S., Juretzek, T., Herchenröder, O., Mogel, M., Poppe, M., Henker, J. and Rethwilm, A. (2004) A simple and rapid single-step multiplex RT-PCR to detect norovirus, astrovirus and adenovirus in clinical stool samples. *J Virol Methods* **118**, 49–59.

Saito, K., Ushijima, H., Nishio, O., Oseto, M., Motohiro, H., Ueda, Y., Takagi, M., Nakaya, S. *et al.* (1995) Detection of astroviruses from stool samples in Japan using reverse transcription and polymerase chain reaction amplification. *Microbiol Immunol* **39**, 825–828.

Sakamoto, T., Negishi, H., Wang, Q., Akihara, S., Kim, B., Nishimura, S., Kaneshi, K., Nakaya, S. *et al.* (2000) Molecular epidemiology of astroviruses in Japan from 1995 to 1998 by reverse transcription-polymerase chain reaction with serotype-specific primers (1 to 8). *J Med Virol* **61**, 326–331.

Walter, J.E. and Mitchell, D.K. (2003) Astrovirus infection in children. *Curr Opin Infect Dis* **16**, 247–253.

Walter, J.E., Mitchell, D.K., Guerrero, M.L., Berke, T., Matson, D.O., Monroe, S.S., Pickering, L.K. and Ruiz-Palacios, G. (2001) Molecular epidemiology of human astrovirus diarrhea among children from a periurban community of Mexico City. *J Infect Dis* **183**, 681–686.

Willcocks, M.M., Ashton, N., Kurtz, J.B., Cubitt, W.D. and Carter, M.J. (1994) Cell culture adaptation of astrovirus involves a deletion. *J Virol* **68**, 6057–6058.

Yan, H., Yagyu, F., Okitsu, S., Nishio, O. and Ushijima, H. (2003) Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. *J Virol Methods* **114**, 37–44.