Human astrovirus diagnosis and typing: current and future prospects

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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.

Currently, there are eight serotypes of H AstV, type 1 being the most prevalent worldwide. Epidemiological observations suggest that H AstV infections do not induce heterotypic immunity, as an episode of H AstV 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 H AstV 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 H AstV 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
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; Willcocks et al. 1994), 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) (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).

### 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

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**Table 1** Most commonly used RT-PCRs for HAstV detection

<table>
<thead>
<tr>
<th>Primers</th>
<th>Genomic region</th>
<th>Length (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mon340/Mon348</td>
<td>ORF1α (pro)</td>
<td>289</td>
<td>Belliot et al. 1997</td>
</tr>
<tr>
<td>A1/A2</td>
<td>ORF1α (C-terminal nsP1a)</td>
<td>192–237</td>
<td>Guix et al. 2002;</td>
</tr>
<tr>
<td></td>
<td>ORF1b (RNA pol)</td>
<td>316</td>
<td>Willcocks et al. 1994</td>
</tr>
<tr>
<td></td>
<td>ORF2 (N-terminal)</td>
<td>413</td>
<td>Noel et al. 1995</td>
</tr>
<tr>
<td></td>
<td>ORF2 (N-terminal)</td>
<td>449</td>
<td>Noel et al. 1995</td>
</tr>
<tr>
<td></td>
<td>ORF2 (C-terminal) + 3′-UTR</td>
<td>296–324</td>
<td>Saito et al. 1995</td>
</tr>
<tr>
<td></td>
<td>ORF2 (C-terminal) + 3′-UTR</td>
<td>1200</td>
<td>Walter et al. 2001</td>
</tr>
<tr>
<td></td>
<td>3′-UTR</td>
<td>89</td>
<td>Mitchell et al. 1995</td>
</tr>
<tr>
<td>Type-specific</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST-S1 to AST-S8, FOR, END</td>
<td>ORF2 (C-terminal)</td>
<td>118–599</td>
<td>Matsui et al. 1998;</td>
</tr>
<tr>
<td>PR6151, PR6257, DM12, JWT4,</td>
<td></td>
<td></td>
<td>Sakamoto et al. 2000</td>
</tr>
<tr>
<td>AST-S5, DM11, Mon2</td>
<td>ORF2 (C-terminal) + 3′-UTR</td>
<td>321–666</td>
<td>Saito et al. 1995;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mitchell et al. 1999;</td>
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<td></td>
<td></td>
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<td>Walter et al. 2001</td>
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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


