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The serotype of adenoviruses detected in faeces by electron microscopy

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Summary

The specific identification of 100 adenoviruses detected in faeces by electron microscopy was attempted using a combination of group-specific and sub-genus F (type 40/41) specific enzyme assays, and isolation in cell culture followed by specific neutralization. Sixty-three were considered to be AdF strains i.e. types 40 or 41 and 37, of which 33 were isolated in cell culture, were deemed to be non-AdF serotypes. The relevance of these results in relation to the diagnosis of viral gastroenteritis is discussed.

Key words: Adenoviruses, serotype, faeces

Introduction

Adenoviruses (AV) of subgenus F (AdF), i.e. types 40 and 41, are probably the second most common cause of juvenile viral gastroenteritis accounting for approximately 10% of all cases[1]. As they are difficult to isolate in conventional cell lines, diagnosis is usually made by the electron microscopical (EM) examination of faeces. Enzyme immunoassays (EIA) for detecting faecal AVs which may be group[2,3] or AdF specific[4] have been developed but their use so far has been limited, probably because of cost. Group-specific EIAs, although potentially able to detect all AV serotypes, would appear to be more efficient in diagnosing infections with AdF, rather than non-AdF, serotypes, probably because AdF strains are usually present in faeces in larger numbers than those commonly isolated in cell culture, e.g. types 1, 2 and 5[2]. It is also possible that commercial EIAs have been developed to have a sensitivity equivalent to that of EM.

Many laboratories examine faeces from diarrhoeic children for the presence of AVs only by EM and no further investigation is usually undertaken on those that are AV-positive. Consequently the serotypes involved are seldom identified but are often assumed to be AdF strains.

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However whilst assessing a latex agglutination test for detecting faecal AVs, it was found that many samples, which were AV-positive by EM, yielded common serotypes in cell culture and were negative for AdF strains. This present study was therefore undertaken to assess the relative proportions of AdF and non-AdF strains detected in faeces by EM and has important epidemiological implications.

Methods

One hundred faeces, AV-positive by EM and stored at 4° C for up to 12 months since collection, were chosen for further study from specimens submitted to the authors' laboratories. Thirty-four samples from Bristol Public Health Laboratory (PHL) had been tested by EM following ammonium sulphate precipitation[5] and graded as occasional, +, ++ or +++, depending on the numbers of AV particles present. The remaining 66 samples, submitted originally to Manchester PHL or Preston PHL, had been concentrated by differential centrifugation prior to EM[6] and were not graded but simply deemed positive.

All samples were examined initially by Adenoclone group (Gp) specific and type 40/41 (i.e. AdF) specific EIAs (Croft Greiner Instruments Ltd.). Those positive by both EIAs were considered to be AdF serotypes and not examined further. Specimens positive by the group-specific EIA but negative by type 40/41 specific or negative by both EIAs were inoculated into HEp2 cells. Isolates were confirmed by EM of cell culture

Table 1. Results of the 100 samples containing adenovirus by EM when examined by group specific and Ad 40/41 specific EIAs and isolation in cell culture

Gp EIA	*40/41 EIA*	*Isolation*	*No.*
+	+	ND*	63
+	–	+	23
–	–	+	10
–	–	–	4
		Total	100

*ND, not done.

fluid when a marked cytopathic effect had developed, usually after a 'blind' passage. Serotyping was carried out by neutralization using antisera supplied by Division of Microbiological Reagents, Central Public Health Laboratory, London.

Results

The findings for the 100 faecal samples are presented in Table 1. Sixty-three were positive by both the group and Ad 40/41 EIAs and were therefore deemed to be AdF strains. From the remaining 37, AVs were isolated in HEp2 cells from 33 (89%); 14 were type 1, nine type 2, two type 3, three type 5, two type 10, and three remained untypable by neutralization. Of those 33 faeces, the Gp EIA was positive with 23 (69%); three reacted very strongly (absorbance > 3.0) and also gave a very weak reaction with the Ad 40/41 EIA, the absorbance being below the cut-off value (i.e. negative control + 0.1) but clearly greater (+ 0.05 to + 0.07) than that of the negative control. Four samples were positive only by EM and negative by all other tests, including isolation: the serotypes of those samples could not be determined.

The 100 samples were analysed by strength of reaction in the Gp EIA (Table 2). The absorbances varied greatly although AdF strains appeared to give significantly higher readings; 45% AdF strains gave readings > 1.0 compared with only 22% of non-AdF serotypes (χ^2 with Yates correction = 4.97, 1df, $0.05 > P > 0.01$.)

Of the 34 specimens, which were graded by EM, 17 were classified as containing + to +++ particles i.e. 10^7–10^{10} ml^{-1} virions approximately. Fifteen of these were considered to be AdF strains while a non-AdF strain was isolated from the other two, both of which were very strongly positive by Gp EIA (vide supra). The remaining 17 samples contained only occasional AV particles by EM i.e. 10^6 ml^{-1} approximately. Seven were considered to be AdF strains, a non-AdF strain isolated from eight, one of which was Gp EIA negative, while the other two were negative by all other tests. The greater proportion of AdF strains in those samples containing + to +++ particles by EM is significant. (χ^2 with Yates correction = 8.3, 1df, $P = < 0.01$).

An attempt was made to correlate the concentration of particles by EM with the EIA absorbance values but no relationship was found.

Discussion

These results clearly show that approximately one third of the AVs detected in faeces by EM may not be AdF strains but other, non-enteric serotypes such as types 1, 2, 3 and 5 which are not proven causes of juvenile gastroenteritis. The clinical significance of AdF indicates that the specific identification of AdF from faecal samples associated with cases of juvenile gastroenteritis should be attempted. This could be done, as in this study, by testing all AVs detected by EM by a specific Ad 40/41 EIA. The EIA used in this study has been shown to be both sensitive and specific in detecting AdF strains (G Kudesia, personal communication) and the finding of a specimen positive by electron microscopy and negative by the Ad 40/41 EIA is indicative of a non-AdF serotype of doubtful clinical significance requiring no further investigation.

The combination of this Ad 40/41 EIA and a sensitive rotavirus EIA[7] would detect the majority of viruses associated with cases of sporadic gastroenteritis, but as the detection rate of adenovirus in faecal samples from cases of sporadic gastroenteritis is only about 10%[1] this may not be considered a cost-effective option, where electron microscopy facilities are available. However the use of such a combination of EIAs could be an alternative where electron microscopy is not available.

Alternatively isolation in Graham 293 cells, which will support the growth of AdF strains could be attempted. This was done by Martin and Kudesia[3] but any isolate would have to be specifically typed by neutralization or restriction endonuclease studies, which are undertaken on a limited basis in only a few reference laboratories, as 293 cells will support the growth of many non-AdF serotypes.[8]

Table 2. Strength of reaction in Gp EIA for the 100 samples according to AV type

	No.	*< 0.1*	*≥ 0.1–0.5*	*> 0.5–1.0*	*> 1.0–2.0*	*> 2.0*
AdF positive	63	0	15(24%)	19(30%)	9(14%)	20(32%)
Non-AdF isolated	33	10(30%)	8(24%)	8(24%)	2(6%)	5(15%)
No AV isolated	4	4(100%)	0	0	0	0
Total	100	14	23	27	11	25

Assessment of results of the 34 samples 'scored' by EM in this study showed that a significantly greater proportion of those with more than the occasional particle were likely to be AdF strains, thus confirming the findings of Brandt et al.[9]. However, as EM is very subjective and operator dependent, reliable identification of AdF strains on this basis is impossible.

It was found that AdF strains tended to give higher readings with the Gp EIA than non-AdF serotypes but as the range of readings with both groups was so wide, accurate differentiation was again impossible.

Of particular interest were the three specimens from which non-AdF strains were isolated and which had high absorbance values (> 3.0) in the Gp EIA with weak absorbance values (0.05–0.07) in the 40/41 EIA. Such samples might represent dual infections where large amounts of group antigen present in the samples interferes in the 40/41 EIA. This is theoretically possible as the 40/41 EIA uses a group-specific monoclonal capture antibody with the AdF specific detecting monoclonal component being peroxidase conjugated. Consequently, excessive group antigen, especially if present in a 'soluble' form from disintegrated virions or disrupted cells, might block all the binding sites, preventing attachment of complete AdF virions and yielding false-negative results. Varying amounts of 'soluble' in relation to virion-associated antigen between specimens might explain the wide range of absorbance values found and the poor correlation between EIA and particle concentration by EM. However a tendency for specimens with high EM counts to have somewhat lower EIA values was also noted.

The significance of non-AdF serotypes in faeces is debatable as they are generally associated with respiratory or ocular disease[10] and not gastroenteritis, which is the usual reason for examining faeces by EM. Furthermore, non-AdF strains may be excreted in the faeces for long periods or intermittently following infection and while this may be important epidemiologically, it may often bear little or no relationship to a patient's current illness. This study has shown that while non-AdF serotypes may be detected by EM they may sometimes be missed by the Gp EIA and by isolation in cell culture.

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References

1 Wadell G, Allard A, Johansson M, Svensson L, Uhnoo I. Enteric adenoviruses. In: *Novel diarrhoea viruses*, Chichester: Wiley (Ciba Foundation Symposium 128) 1987; 63–91
2 Bryden AS, Chesworth C. An assessment of Adenoclone EIA; a monoclonal-based group-specific enzyme assay for adenovirus diagnosis. *Serodiagn Immunother Infect Dis* 1988; **2**: 341–7
3 Martin AL, Kudesia G. Enzyme linked immunosorbent assay for detecting adenoviruses in stool specimens: comparison with electron microscopy and isolation. *J Clin Pathol* 1990; **43**: 514–15
4 Wood DJ, Bijlsma K, de Jong JC, Tonkin C. Evaluation of a commercial monoclonal antibody-based enzyme immunoassay for detection of adenovirus types 40 and 41 in stool specimens. *J Clin Microbiol* 1989; **27**: 1155–8
5 Caul EO, Ashley CR, Egglestone SI. An improved method for the routine identification of faecal viruses using ammonium sulphate precipitation. *FEMS Microbiol Letters* 1978; **4**: 1–4
6 Flewett TH, Bryden AS, Davies HA. Diagnostic electron microscopy of faeces. 1. The viral flora of the faeces as seen by electron microscopy. *J Clin Pathol* 1974; **27**: 603–8
7 Bryden AS. The immunological diagnosis of rotavirus. *Serodiagn Immunother Infect Dis* 1990; **4**: 407–11
8 Yirrell DL, Roome APCH, Darville JM, Ashley CR, Harbour J. Comparison of the continuous cell line 293 with human embryo kidney cells and human embryo fibroblast cells for the cultivation of ocular viruses. *J Clin Pathol* 1983; **36**: 996–9
9 Brandt CD, Rodriguez WJ, Kim HW, Arrobio JO, Jeffries BC, Parrott RH. Rapid presumptive recognition of diarrhea-associated adenoviruses. *J Clin Microbiol* 1984; **20**: 1008–9
10 Stott EJ, Garwes DJ. Rhinoviruses, adenoviruses and coronaviruses: their role in respiratory disease. In: Collier LH, Timbury MC eds. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, Vol. 4 Virology 8th edn. London: Edward Arnold, 1990; 244–72