Recent respiratory and enteric adenovirus infection in children in the Manchester area

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Summary

Seventy-three group B adenoviruses (29 type 3 and 44 type 7) identified in a recent community outbreak were analysed with restriction endonucleases. Considerable genetic heterogeneity was identified, particularly amongst the type 3 isolates, but this genome variation could not be correlated with either clinical or epidemiological findings. Group F adenoviruses were found in 132 (4.1%) of 3202 stool specimens from children with gastroenteritis and, after rotaviruses, they were the most common viruses identified. Unlike rotaviruses, these enteric adenoviruses were endemic throughout the 3-year study period and the greatest proportion of infections (47.6%) were found in babies under 6 months old.

Introduction

Three clinically and epidemiologically distinct patterns of adenovirus infection can be recognized in children, each caused by a different group of adenoviruses. Types 1, 2, 5 and 6 (group C) are endemic in children under 5 years old. They are a common cause of mild respiratory tract infection, and prolonged asymptomatic shedding of these viruses in the stools frequently occurs after primary infection. Adenovirus types 3 and 7 (the commonest members of group B) cause community epidemics of respiratory and ocular disease every few years; they are particularly severe respiratory pathogens in children under one year, when infection is occasionally fatal, or it may cause permanent pulmonary damage[1]. In contrast, adenovirus types 40 and 41 (group F) are enteric rather than respiratory pathogens and they are an important cause of infantile gastroenteritis[2]. Group B and C adenoviruses are usually diagnosed by isolation of the virus in cell culture, followed by identification of the serotype by neutralization tests, but group F adenoviruses grow poorly in conventional cell cultures and are recognized by electronmicroscopy (EM) of the stools.

This report describes recent infections due to groups B and F adenoviruses in children in the Manchester area. The genetic variation amongst adenovirus types 3 and 7 isolated during a recent community epidemic was identified by restriction endonuclease (RE) analysis, and correlated with clinical and epidemiological findings. The aims were (*a*) to see whether genetic variation occurred during the course of the epidemic; and (*b*) to examine the suggestion that particular genome variants may be associated with particular clinical presentations[3]. Secondly, the pattern of adenovirus F infection in children with gastroenteritis was studied over a 3-year period. An immune EM technique was introduced for definitive diagnosis of these viruses, and their incidence and seasonality was compared with that of rotavirus infections over the same period.

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Methods

Source of viruses

Group B adenoviruses (44 type 7 and 29 type 3) recovered from patients referred to hospitals within the catchment area of the North Manchester Regional Virus Laboratory (NMRVL) between August 1983 and February 1985 were studied. Techniques for virus isolation and identification have already been described[4]. Group F adenoviruses were identified by EM of stool specimens from children with gastroenteritis submitted to the NMRVL between July 1983 and March 1986. Faecal extracts of these stool specimens were also inoculated into the 4 cell lines (human embryo fibroblasts, rhesus monkey kidney, Vero and HEp-2 cells) used routinely for virus isolation at the NMRVL. Between July 1983 and June 1984, a presumptive diagnosis of adenovirus F infection was made if adenovirus particles were seen by EM which could not be recovered in cell culture. After July 1984, all adenoviruses seen by EM were re-examined by an immune EM technique which definitively distinguishes the enteric adenoviruses from other adenovirus serotypes[5]. Other viruses seen by EM in the stool specimens (viz., rotavirus, astrovirus, coronavirus, calicivirus and Norwalk-like viruses) were identified by morphological criteria only.

RE analysis of adenovirus genomes

For this analysis, ^{32}P-labelled adenoviruses were grown in Graham 293 cells[6] in wells of microtitre plates, and restriction enzymes *Sma* I, *Bam* HI, *Pvu* II, *Sst* II, *Bgl* I, *Pst* I, *Hind* III and *Bst* II (Bethesda Research Laboratories, Paisley, Scotland) were used as previously described[4]. All 77 group B adenovirus isolates were analysed with all the above enzymes; in addition, the DNA of 74 group F adenoviruses was analysed by digestion with *Sma* I and *Hind* III.

Results

Group B adenoviruses

The epidemic pattern of the 73 adenovirus types 3 and 7 are shown in Figure 1, and contrasted with the recovery of the endemic adenoviruses types 2 and 5 from clinical material submitted to the NMRVL over the same period of time. The peak of the epidemic of both types 3 and 7 occurred in April 1985.

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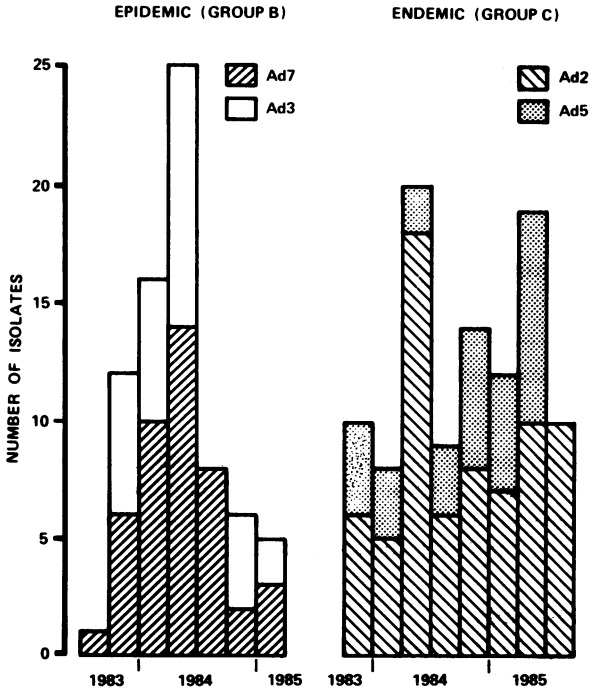

Figure 1. Epidemic pattern of adenovirus types 3 and 7 isolated at the NMRVL between July 1983 and June 1985, compared with the endemic pattern of adenovirus types 2 and 5 that were recovered over the same period of time

Table 1 summarizes the clinical presentation and age distribution of patients infected with adenovirus types 3 and 7; 59 (81%) of these viruses were recovered from children under 15 years, the majority of whom were under 5 years. There was one fatality, a 9-month-old girl infected with adenovirus type 7 who died of pneumonia and encephalitis. Adenovirus type 3 was rarely recovered from children under one year; otherwise clinical presentations of the two viruses were similar, with the majority of infections presenting as acute respiratory disease or pharyngoconjunctival fever in children, but as follicular conjunctivitis in older patients.

Considerable genetic variation was identified in these group B adenoviruses and detailed DNA cleavage patterns obtained have already been described[4]. Approximately similar numbers of three different type 7 genotypes were found, all variants of the previously described type 7b variant[7], and nine distinct type 3 genotypes were identified. However, the distribution of these variants did not alter during the course of the epidemic, neither were particular genome variants associated with particular clinical presentations.

Table 1. Clinical presentation and age distribution of 29 patients with adenovirus type 3 and 44 patients with adenovirus type 7 infections

Clinical presentation	*No. of patients of age (in years)*			
	<1	*1-4*	*5-14*	*>14*
RTI	5●	20	4	2
PCF	5	7	1	
FC				10
Other	2	10	5	2

RTI, respiratory tract infection; PCF, pharyngoconjunctival fever; FC, follicular conjunctivitis; other, predominant symptoms diarrhoea and/or pyrexia
●One 9-month-old girl died of adenovirus type 7 pneumonia and encephalitis

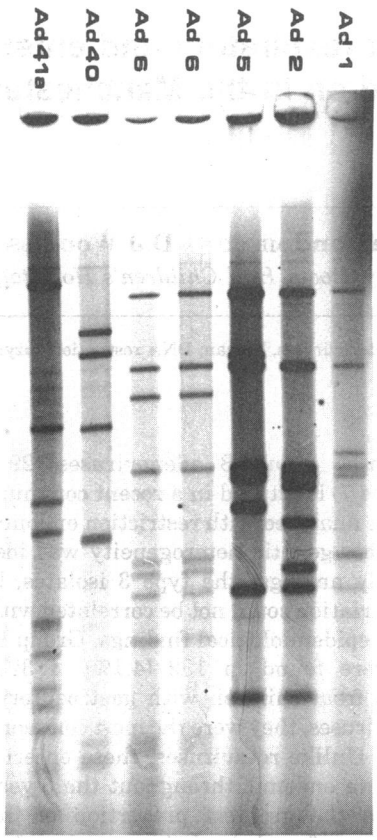

Figure 2. DNA cleavage patterns of adenoviruses frequently present in stool specimens from young children obtained by digestion with Hind *III. Group F adenoviruses (types 40 and 41a) can both be clearly distinguished from Group C adenoviruses (types 1, 2, 5 and 6). (Two lanes of adenovirus type 6 are shown)*

Group F adenoviruses

Adenoviruses were seen by EM in 186 (5.8%) of 3202 stool specimens, and 132 (4.1%) were identified as group F (46 presumptive and 86 definitive). Confirmation of the specificity of the immune EM technique was obtained when 74 viruses identified as group F by immune EM were analysed with REs and identified as type 40 (15 viruses) or type 41a (59 viruses)[8]. A comparison of the RE analysis of these two viruses and types 1, 2, 5 and 6 (which are also often recovered from stools of young children) is shown in Figure 2.

Group F adenoviruses comprised 15.7% of all the viruses detected in the stool specimens, and they were second in prevalence to rotaviruses (Table 2). Unlike the rotavirus infections, which occurred

Table 2. Viruses identified in 3202 stool specimens from children with gastroenteritis

Virus	*Number*	*(%)*
Rotavirus	599	(18.7)
Adenovirus group F	132	(4.1)
Other adenoviruses	54	(1.7)
Other viruses●	57	(1.8)

●Astrovirus (26), calicivirus (12), Norwalk-like virus (10), coronavirus (9)

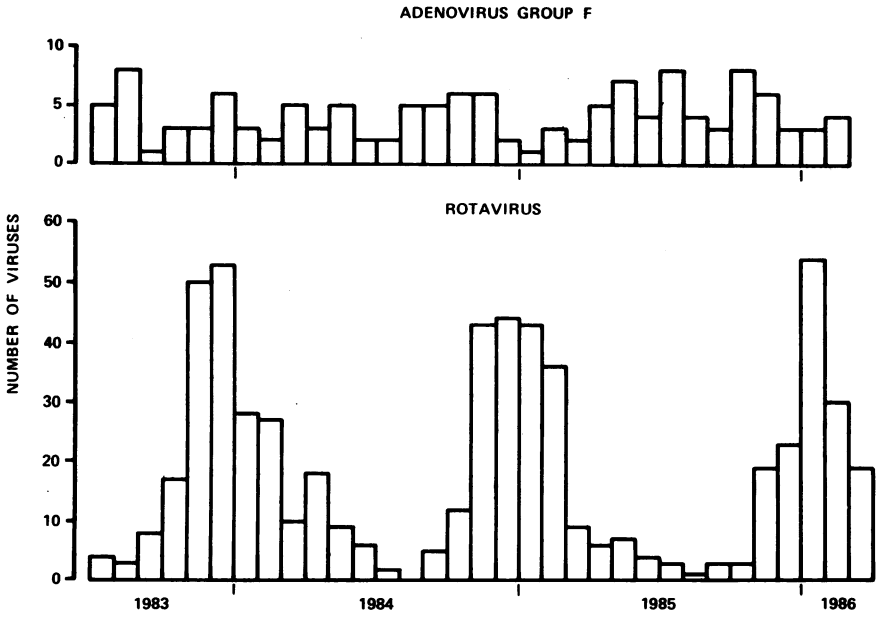

Figure 3. Endemic pattern of group F adenoviruses identified at NMRVL between July 1983 and March 1986, compared with annual winter epidemics of rotavirus infection

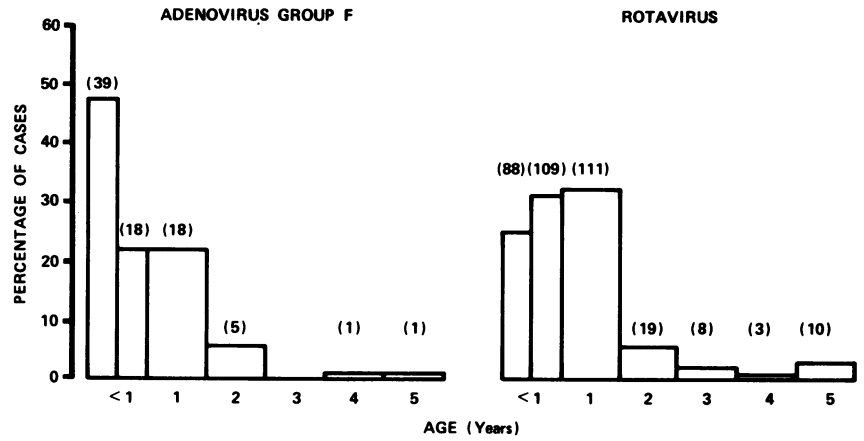

Figure 4. Age distribution of children with gastroenteritis due to group F adenoviruses and rotaviruses between July 1984 and March 1986. Figures in brackets show total number of viruses in each age group

predominantly as annual winter epidemics, group F adenoviruses appeared to be endemic in Manchester throughout the period of study (Figure 3). The age distribution of children from whom group F viruses were recovered also differed significantly from children with rotavirus infection. The peak incidence of group F adenovirus infection occurred in children under 6 months old, whereas the peak incidence of rotavirus infection was at 13-24 months (Figure 4). Moreover, the proportion of children under 6 months old with group F adenovirus infection was significantly greater than the proportion with rotavirus infection in the same age group ($\chi^2_{(1)}$ with Yates's correction=14.8, P=0.0003).

Discussion

The recent epidemic of adenovirus type 3 and 7 infection in Manchester provided the opportunity to investigate the extent of genetic variation that was occurring in a community outbreak of group B adenovirus infection. It also allowed us to investigate the possibility that particular adenovirus genome variants were associated with particular clinical syndromes, as has been suggested by Wadell[3]. Although considerable genetic variation was identified, particularly amongst type 3 isolates, this variation did not appear to affect clinical presentation, neither was there any evidence of genetic instability of the variants identified during the course of the epidemic. Comparable genetic variation was identified in a community outbreak of follicular conjunctivitis due to adenovirus type 3 in Glasgow in 1981, when six different genome variants were identified in 38 isolates by O'Donnell and co-workers[9]. As in our study, these authors were unable to correlate differences in clinical presentation with differences in the viral genome. The significance of the genetic heterogeneity of these group B adenoviruses therefore remains obscure. However, RE analysis provides a powerful new tool for studying the epidemiology of adenovirus infection. Long-term studies will provide useful data on the genetic stability of the genome variants and the relevance of this to epidemics of group B adenovirus infection.

Group F adenoviruses are now firmly established as causative agents of gastroenteritis in young children[2]. This study, in common with those of Uhnoo[2] and Madeley[10], shows that these agents are,

after rotaviruses, the most frequent virus identified in stools of children with gastroenteritis. Unlike rotaviruses, they appear to be endemic rather than epidemic; the reason for this different seasonal distribution of two viruses, both presumably spread by the faecal-oral route, is not known.

The different age distribution of children with gastroenteritis due to rotaviruses and group F adenoviruses, which has also been noted in one other study[11], may be because enteric adenovirus infection is actually commoner than rotavirus infection in babies under 6 months old. Alternatively, it may be because asymptomatic rotavirus carriage is common in this age group[12]. Whichever the case, this study shows that enteric adenovirus infection is a particularly important cause of morbidity due to gastroenteritis in children under 6 months old.

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