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Similarities in nucleotide sequence between serum and faecal human parvovirus DNA

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SUMMARY

Two DNA clones were obtained from faecal specimens containing a parvovirus-like small round virus from a 1977 outbreak of gastroenteritis, and their nucleotide sequences were determined and found to be essentially identical with parts of the published sequence of serum parvovirus B19 and with a B19 isolate (JB) partially sequenced in this study. The clones corresponded mainly to genome regions coding for non-structural proteins, but also include a sequence of some 160 bp coding for structural proteins. Southern blotting experiments with a full-length B19 probe revealed a virion-sized 5·5 kbp DNA band in specimens from gastroenteritis cases in both 1977 and 1986. Thus the nucleotide sequence and hybridization results suggest that the virus seen in these studies is very similar to B19. Further work is necessary to clarify the antigenic relationship of these viruses.

Parvovirus-like particles have been observed in human faeces, and implicated in the aetiology of gastroenteritis [1–5], but where these have been tested they were found to be antigenically distinct from the serum parvovirus B19 [2, 6]. B19 is the causative agent of erythema infectiosum, amongst a range of disease manifestations [7]. The extent of sequence divergence in the genome of this virus has previously been studied by the restriction enzyme mapping of more than 50 virus isolates [8, 9]. This indicated that these isolates are very similar, even when they originate from distant sources, date from different years, and are associated with dissimilar clinical manifestations. The limited sequence divergence observed by restriction mapping has been confirmed by the sequencing of two isolates (B19-Au and B19-Wi) which show > 99% homology [10, 11]. The source of virus for all these studies has been human serum in which, during the viraemic phase of B19 infection, virus particles are abundant.

A virus present in faeces was observed during a large countrywide outbreak of gastroenteritis associated with consumption of shellfish [4]. The characteristics of this virus on caesium chloride gradient centrifugation and subsequent electron microscopy (EM) suggested that it was a parvovirus, but serologically unrelated to B19. Experiments were undertaken to clone the genome of the virus so that its

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Table 1. *Comparison of nucleotide sequences**

Restriction endonuclease changes relative to B19-Au	Nucleotide	Faecal parvovirus	B19-Wi	B19-Au	Amino acid changes relative to B19-Au
	254	C	T	T	In predicted non-coding region
	272	C	C	T	
	330	C	G	C	
	333	C	A	T	
	418	G	A	A	
	430	A	G	C	
Xba I site lost	480	G	G	A	—
	577	C	T	T	—
	692	T(ile)	T(ile)	C(thr)	ile-thr
	723	C	C	T	—
	2352	G	G	A	—
	2453	G(glu)	G(glu)	A(lys)	glu-lys
	2527	A	G	A	—
	2575	C	T	T	—
	2594	A(asn)	C(his)	A(asn)	his-asn

* Nucleotides 162–805, 1867–2100 and 2271–2600; all other bases in these regions were identical. Sequence data on B19-Au and B19-Wi were from Shade et al. [10] and Blundell et al. [11]; nucleotide numbering and reading frame assignments are according to those authors.

relationship to other human parvoviruses might be better understood. The strategy chosen to clone the viral DNA was essentially standard cDNA methodology [12] based on the assumption that the genome was single-stranded (ss) DNA with a structure similar to that of all other characterized parvoviruses [13]. That is, extracted DNA would be either ss of one predominant polarity, like most autonomous parvoviruses, or double-stranded (ds) due to annealing of separately encapsidated single strands of opposite polarity, like the adeno-associated viruses and B19.

A 10% suspension of faeces was extracted with trichlorotrifluorethane and its components separated by centrifugation on caesium chloride [3]. The fractions with a buoyant density of 1·38–1·41 g/ml contained large numbers of EM-detectable 22–25 mm diameter particles identical in appearance to parvoviruses. Apart from short sections of bacterial flagellae, little debris was observed. These fractions were pooled, diluted and pelleted through a sucrose cushion (12·5% sucrose; 35000 rev./min.; 2 h; Beckham SW41 rotor). Nucleic acid was extracted from the pellet with proteinase K, SDS and phenol/chloroform, and was then incubated with Klenow polymerase and nucleotides to complete synthesis of the second DNA strands. This step was intended to convert any ss DNA to a ds form by priming from terminal hairpin structures. Subsequently, the ds DNA was treated with nuclease S1 to generate blunt ends suitable for cloning. The DNA was then extended with deoxycytidine homopolymer tails, and linked with deoxy-guanidine tailed pBR322 which had been cleared at the *Pst* I site. Following transformation of *Escherichia coli* HB101, 405 colonies were screened and two were found to contain inserts at the plasmid *Pst* I site (pD14 and pD17). Unexpectedly,

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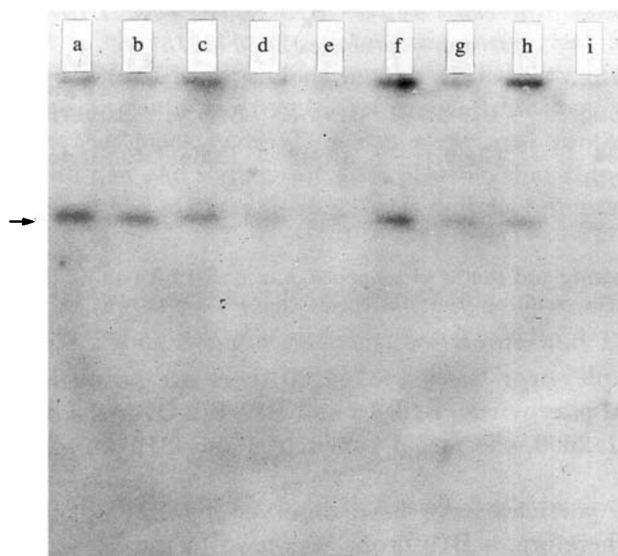

Fig. 1. Samples from two outbreaks of gastroenteritis (a–e and i from 1977; f–h from 1988) analysed by DNA hybridization. Nucleic acid was extracted from 10% faecal suspensions by phenol extraction and ethanol precipitation. DNA was run on a 1% agarose gel which was transferred to a nylon membrane by southern blotting. The blot was probed with 10^8 c.p.m. ^{32}P-labelled B19 probe and autoradiographed [9, 16]. Unlabelled *Hind*d III-digested λDNA was used as a molecular weight marker and was detected by ethidium bromide staining of the gel prior to blotting. The arrow indicates the position of the 5·5 kb band.

pD14 and pD17 were found to hybridize with B19 DNA. Therefore, dideoxy sequencing from the inserts was undertaken after subcloning them into M13 mp18 and mp19 vectors. The pD17 insert is approximately 900 nucleotides (nt) and starts at nt 160 in the published B19 sequence [4, 5]. The pD14 insert is approximately 1000 nucleotides and starts at nt 1867 in the published B19 sequence. These two clones correspond mainly to genome regions coding for non-structural proteins, but also include some 160 nts of structural protein sequence (pD14). As only these two clones, representing 1·9 kb of an expected 5·5 kb genome, were obtained, it was not possible to sequence the whole genome. Specifically, it was not possible to obtain sequence information in the putative structural protein-coding region of the faecal parvovirus genome which might have shed light on the apparent antigenic differences between this virus and B19. Table 1 shows the nucleotide differences between the faecal parvovirus, B19-Au and B19-Wi [10, 11]. There are 12 nucleotide differences between the faecal parvovirus and B19-Au over 1205 bases (99% homology), 5 of which are in a predicted non-coding region. Of the remainder, only two would result in amino acid changes (*ile* instead of *thr* at 692, *glu* instead of *lys* at 2453). Between faecal parvovirus and B19-Wi there are only nine base differences (> 99% homology) over the 1205 bases, only one of which would result in an amino acid change (*asn* instead of *his* at 2594). The faecal parvovirus and B19-Wi both differ from B19-Au at position 480 (G instead of A), explaining their lack of an *Xba* I site at this position [9]. Some regions of the genome appear to show greater variation than

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Table 2. *Nucleotide differences between B19-JB, B19-Au, B19-Wi and faecal parvovirus in bases 2430–2575*

Nucleotide*	B19-JB	B19-Au	B19-Wi	Faecal parvovirus
2453	G(glu)†	A(lys)	G(glu)	G(glu)
2515	A	G	G	G
2527	A	A	G	A
2575	T	T	T	C

* Nucleotide numbering and source of sequence data on B19-Au and B19-Wi as in Table 1.
† Amino acid changes resulting from nucleotide changes are shown.

others. For example, over bases 1867–2100 there are no nucleotide differences between the faecal parvovirus, B19-Au and B19-Wi. Over the other stretches, nt 162–805 and 2271–2600, the faecal parvovirus and B19-Au showed 98·6% and 99·1% homology.

Parvovirus-like particles have been observed by EM in other outbreaks of gastroenteritis. Therefore, a B19 probe was used to hybridize with nucleic acid extracted from stool samples from outbreaks of gastroenteritis in the UK in 1977 and 1986 (Fig. 1). A band of about 5·5 kb was observed in all samples. This is the same size as the B19 genome, and thus supports the idea that this DNA came from viral particles and not from bacterial chromosomal DNA, which would migrate as a smear near the origin of the gel. Also, under the same conditions plasmid vector DNA did not hybridize to the samples, further ruling out a bacterial origin for the bands. As a further control, parvovirus H1 cloned DNA (gift of Dr S. Rhode III) was used as a probe and no signal was observed. The stool samples were previously examined by EM, and numbers of parvovirus-like particles were observed. No other viruses were detected by EM or culture. In contrast, however, samples from cases of gastroenteritis containing other viruses such as rotavirus, adenovirus and small round structured viruses of the Norwalk group did not hybridize with B19 DNA.

For further characterization of the extent of genomic diversity of B19 a small region of B19-JB cloned in pGEM-1 [9, 14] and subcloned into M13 was sequenced (145 bases). Over this region it differed from the other isolates at four positions only; there were only two differences from any particular isolate (98·6% nt homology, Table 2), indicating that all four genomes show a similar degree of sequence divergence from one another.

Two of the virus isolates discussed here were from sickle-cell anaemia aplastic crisis cases (B19-Au and B19-JB), and one was from an asymptomatic blood donor (B19-Wi). The other virus was from a case of gastroenteritis (faecal parvovirus). The homology between the faecal parvovirus DNA and the B19 isolates is unexpected, since B19 and the faecal parvovirus were thought to be serologically distinct, and there appear to be a number of different antigenic strains of the faecal viruses [4–6]. Also, in an experimental B19 infection of human volunteers, B19 DNA was not detectable in stool samples [15]. Nevertheless, it is not inconceivable that B19 virus could be shed in the faeces, given that up to 40% of individuals with B19 erythema infectiosum experience gastrointestinal symptoms [7]. It could be argued that the faecal virus is a B19 isolate, since it shows 99%

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nucleotide homology to B19-Au, even though no serological relationship has been demonstrated. It is clear that more data from serological and hybridization studies are needed, which is dependent on the availability of serum and faecal specimens from appropriate gastroenteritis cases. Additionally, it is important that clones from the genes coding for the structural proteins of the faecal parvovirus are obtained and sequenced. It is possible that these genes may show more variation than that of the non-structural regions sequenced in this study.

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