Journal of General Virology (2000), **81**, 1017–1025. Printed in Great Britain

Integrity and full coding sequence of B19 virus DNA persisting in human synovial tissue

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Primary infection by human parvovirus B19 is often accompanied by arthropathy of varying duration, of which the most severe cases can be indistinguishable from rheumatoid arthritis (RA). While this might seem to imply a role in RA pathogenesis, recent studies have verified long-term persistence of B19 DNA in synovial tissue not only in patients with rheumatoid or juvenile arthritis, but also in immunocompetent, non-arthritic individuals with a history of prior B19 infection. However, the latter data are based on PCR amplification of short segments of DNA, with little sequence information. We determined the nucleotide sequence and examined the integrity of the protein-coding regions of B19 genomes persisting in synovial tissue and compared the results with data from synovial tissues of recently infected patients. In synovium of both previously and recently infected subjects, the viral coding regions were found to be present in an apparently continuous, intact DNA molecule. Comparison with sequences reported from blood or bone marrow showed that the synoviotropism or persistence of the B19 virus DNA was not due to exceptional mutations or particular genotype variants. The synovial retention of full-length viral genomes may represent a physiological process functioning in long-term storage of foreign macromolecules in this tissue.

Introduction

Parvovirus B19 is the smallest DNA virus known to cause disease in humans. The viral genome is single-stranded DNA of 5596 nt and has identical 383 nt inverted terminal repeats at each end (Deiss *et al.*, 1990). The 3′ side of the negative-polarity genome encodes the non-structural protein NS1 and the 5′ side the two capsid proteins VP1 and VP2. The genes for the structural proteins overlap, so that the VP2 open reading frame is included totally within that of VP1, with the VP1 gene encoding an additional 227 amino acids unique to the VP1 N terminus (Shade *et al.*, 1986; Ozawa *et al.*, 1987; Cotmore *et al.*, 1986).

The nucleotide sequence of the entire B19 genome has been determined only from blood, in an asymptomatic donor (Blundell *et al.*, 1987) and in a child with aplastic crisis (Shade *et al.*, 1986). Shorter fragments have also been sequenced from other sources (Erdman *et al.*, 1996; Umene & Nunoue, 1993, 1995), but no disease-specific sequence variations have been observed (Umene & Nunoue, 1993; Morinet *et al.*, 1986; Mori *et al.*, 1987; Haseyama *et al.*, 1998; Hemauer *et al.*, 1996; Nguyen *et al.*, 1999). In general, the unique part of VP1 is the most variable region in the B19 genome.

B19 infection is prevalent; more than half of the adults in industrialized countries have anti-B19 IgG antibodies (Cossart *et al.*, 1975; Anderson *et al.*, 1986). The virus is the major aetiological agent of aplastic crisis, complicating haemolytic anaemias of various forms (Anderson *et al.*, 1982; Serjeant *et al.*, 1981; Pattison *et al.*, 1981), and is responsible for erythema infectiosum (fifth disease), a common epidemic disease in children and young adults (Anderson *et al.*, 1984). During pregnancy, B19 can be transmitted from mother to foetus, whereby the infection can lead to hydrops and foetal death (Brown *et al.*, 1984; Miller *et al.*, 1998). Although the infection in adults is often asymptomatic, especially among females it is frequently complicated by post-infectious arthropathy (Reid *et al.*, 1985; White *et al.*, 1985), typically affecting small joints. In

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The GenBank accession numbers of the sequences reported in this study are AF161223–AF161228.

most cases, parvovirus arthropathy is transient, but it can become chronic and fulfil the diagnostic criteria of rheumatoid or juvenile arthritis (Naides, 1993).

The host cells for B19 virus replication in bone marrow are erythrocyte precursors CFU-E and BFU-E (Ozawa *et al*., 1986; Srivastava & Lu, 1988). The cellular receptor has been identified as the glycolipid globoside (Brown *et al*., 1993), while certain other glycosphingolipids may have a similar function (Cooling *et al*., 1995). The tissue distribution of these surface receptors correlates with B19-associated disease, even though virus replication is restricted to the erythroid lineage (Brown *et al*., 1993; Cooling *et al*., 1995). Persistence of B19 DNA in blood and/or in bone marrow due to ongoing virus replication is well established in immunodeficiency (Kurtzman *et al*., 1988). However, B19 persistence in bone marrow has also been described in some immunocompetent individuals with parvovirus-related symptoms, and occasionally without an apparent clinical association (Nikkari *et al*., 1995; Lundqvist *et al*., 1999).

In synovial tissue, B19 DNA has been shown to persist for years or decades after primary infection in a large proportion of subjects with or without chronic arthropathy (Saal *et al*., 1992; Kerr *et al*., 1995; Söderlund *et al*., 1997; Cassinotti *et al*., 1997). However, the synovial persistence data have been based on PCR amplification of segregated, relatively small regions of viral DNA. For example, the previous study from our group utilized primers spanning a 1066 bp fragment at the junction area of the non-structural (NS1) and capsid protein (VP1 and VP2) genes, i.e. one-fifth of the B19 genome (Söderlund *et al*., 1997). Neither the molecular and cell-biological mechanisms nor the possible clinical implications of synovial B19 DNA persistence are known.

In order to assess whether B19 DNA occurs in the synovial tissue as an intact molecule or in fragments, we performed PCRs at three different sites of the viral genome using DNA preparations diluted to the end-point as the template. To assess whether the synovial B19 is of a unique genotype or involves particular codon changes that could be causally related to tissue tropism or persistence, we amplified and sequenced 97% of the protein-coding region of the viral genome from four subjects with B19 carriership of varying duration and obtained partial sequence data from a fifth subject. The results were compared with each other and with previously reported B19 sequence data.

Methods

■ **Serum and tissue samples.** Samples of synovial tissue were obtained during arthroscopy by using basket forceps or a synovial shaver from 30 constitutionally healthy adults (age span 18–53, mean 21 years) with joint trauma or exertion. Sera were also obtained for B19 antibody testing. These subjects are additional to those studied before (Söderlund *et al*., 1997). The sampling occurred with the patients' informed consent at the Finnish Central Military Hospital and the study was approved by the Ethics Committee of the Finnish Defence Forces.

■ **Antibody assays.** Anti-B19 IgG antibodies in serum were measured by a commercial enzyme immunoassay (EIA) (Dako) and anti-B19 IgM antibodies by the EIA of Biotrin. For further evidence of the time of B19 primary infection, the sera of six subjects examined in depth for synovial B19 DNA were studied for epitope-type specificity (ETS) of anti-VP2 IgG (Söderlund *et al*., 1995), as described recently (Kaikkonen *et al*., 1999). An 'acute' pattern in this assay indicates B19 primary infection within 3–6 months, whereas a 'non-acute' pattern indicates B19 immunity of longer duration.

■ **DNA purification.** DNA was purified by proteinase K digestion followed by phenol extraction and ethanol precipitation (Söderlund *et al*., 1997), but using glycogen as a carrier.

■ **DNA controls.** DNA from a viraemic serum containing 10^{11} B19 genomes per ml was included as a positive control at a dilution of 10^{-5} in all PCR assays. Water was used as a negative control. Because of the high sensitivity of PCR, extreme precautions were taken to avoid false-positive results. Sample handling, in laminar-flow hoods, and preparation of the reaction mixtures occurred in isolated rooms. Aerosol-resistant pipette tips and disposable racks were used to avoid carry-over. The tissue samples were shown to be negative for PCR inhibitors by amplification of the human chromosomal gene for glyceraldehyde-3-phosphate dehydrogenase. For further controls, tissues from 12 B19-seronegative individuals were processed identically in the VP1 PCR assay, with no positive results.

■ **Primer design and PCR optimization.** Primers were designed according to the sequence of the Au isolate (Shade *et al*., 1986) and our nucleotide numbering corresponds to this reference sequence. Optimal reaction conditions for each primer set were determined by using the control DNA as template.

We aimed to equalize the sensitivities of the NS1 and VP2 PCR methods to the level of the VP1 PCR, which has been shown to detect one target molecule in 2 µl template (Söderlund *et al*., 1997). The sensitivities of the assays were monitored with serially diluted control DNA. The detection sensitivities of all three PCRs were within one order of magnitude.

To compare the different B19 genomic regions within individual samples, DNA suspensions were diluted serially in 10-fold steps and each dilution was studied by the nested VP1 PCR. The last dilution (end-point) giving a positive signal by ethidium bromide staining was then used as a template in the NS1 and VP2 PCRs (Table 2).

■ **VP1 PCR.** All the tissue samples were first studied with a nested PCR (VP1 PCR), as described by Söderlund *et al*. (1997). This PCR amplifies a 1066 bp region in the middle of the genome, spanning the junction between the nonstructural (NS1) and structural (VP1 and VP2) genes. For further analysis of B19 DNA, we selected two patients with serological evidence for recent B19 infection (IgG^+, IgM^+ and ETS^+) and chose four subjects randomly with long-term B19 carriership (IgG^+, IgM^- and ETS^-).

■ **NS1 PCR.** The outer primer pair was NSofwd and NSorev and the inner primer pair was NSifwd and NSirev (Table 1). Two µl of the end-point-diluted DNA suspension was added to 48 µl PCR mixture [0·2 µM both primers, 2·5 U Ampli*Taq* Gold (Perkin Elmer), 200 µM each nucleotide in Perkin-Elmer PCR buffer] and, after pre-heating at 94 °C for 10 min and 30 PCR cycles (94 °C for 30 s, 57 °C for 30 s, 68 °C for 1·5 min), 2 µl product was added to 48 µl reaction mixture with the inner

Table 1. Primers used in this study

The location of each primer within the sequence of the B19 genome is shown.

Primer	Sequence	Location
VP1 PCR		
p6	GGAGAATCATTTGTCGGAAG	2086–2105
p3	CTTCTGCAGAATTAACTGAAGTC	3149–3127
p8	TGTGCTTACCTGTCTGGATTG	2407–2427
p5	AGGCTTGTGTAAGTCTTCAC	2797–2777
p1	ATGAGTAAAGAAAGTGGCAAATGG	2444–2467
NS1 PCR		
NSofwd	ATGGAGCTATTTAGAGGGGTG	436–456
NSorev	TTTGCAATCCAGACAGGTAAGC	2431–2410
NSifwd	ACTGGTTGTGTGAAAACAGAGTG	1097–1119
NSirev	TTTTCCTGCTACATCATTAAATGG	1533–1510
NSpfwd	AGAGGATAAGTGGAAACTAGTTG	1125–1147
NSprev	TACTGGAACACTTTTAGCAATGG	1476–1454
VP2 PCR		
VP2ofwd	TGACTTCAGTTAATTCTGCAGAAG	3126–3149
VP2orev	TGGGTGCACACGGCTTTTGG	4783–4764
VP2ifwd	CTAGAATATCCTTACGCCCTGG	4095–4116
VP2irev	GTGGCTGATGCAAACCCCATC	4481–4461
VP2pfwd	CCATTTCTCATGGTCAGACCAC	4176–4197
VP2prev	CCATACAGAACCCACCATTAGG	4369–4348
Sequencing		
rt1	AATTTAGAGGGCTGCAGTCAAC	3845–3866
NSsfwd	GCAATGGCCATTGCTAAAAGTG	1447–1468
p9	CTTTAGGTATAGCCAACTGG	2905–2924
rtsrev	ATGTGTCAGGAACCCCTAAGC	3923–3903

primers (0·4 μM NSifwd, 0·8 μM NSirev, 200 μM each nucleotide and 2·5 U Ampli*Taq* Gold in PCR buffer). DNA was amplified for another 40 cycles (94 °C for 30 s, 57 °C for 30 s, 72 °C for 1·5 min).

The second-round products were detected by agarose gel electrophoresis and ethidium bromide staining. In addition, the products were transferred to a nylon membrane and hybridized with an NS1-specific, digoxigenin-labelled probe. The probe was made by PCR with primers NSpfwd and NSprev (Table 1).

■ **VP2 PCR.** Two μl of the end-point-diluted DNA suspension was used as a template for the first-round PCR (pre-heated at 94 °C for 10 min, 30 cycles of 94 °C for 15 s, 57 °C for 30 s, 68 °C for 1·5 min), performed with 0·2 μM of each of the outer primer pair VP2ofwd and VP2orev. Two μl of the product was transferred to 48 μl reaction mixture with 0·4 μM inner primers VP2ifwd and VP2irev (Table 1). Conditions for the 40 cycles of the nested reaction after preheating were 94 °C for 30 s, 57 °C for 30 s and 72 °C for 20 s. Again, the products were detected with electrophoresis followed by Southern blotting. The probe was amplified with primers VP2pfwd and VP2prev (Table 1).

■ **Duplex PCR.** Using end-point-diluted DNA as the template, both the VP2 and NS protein-coding sequences were amplified simultaneously in one tube. The first-round PCR utilized the outer primer pairs of the VP2 and NS reactions. The product was purified with the High Pure PCR product purification kit (Boehringer Mannheim) and eluted with 100 μl sterile water, of which 4 μl was transferred to the second reaction tube. Primers for the nested reaction were NSifwd, NSirev, rt1 and VP2irev (Table 1). The primer concentrations were 0·5 μM except for NSirev, for which the optimal concentration was found experimentally to be 1 μM. The resulting amplicons were 439 and 639 nucleotides in size, respectively, and they were separated electrophoretically on a 1% TAE–agarose gel and Southern blotted. Hybridization was done simultaneously with NS1 and VP2 probes.

■ **Sequencing.** B19 DNA purified from the synovium of five subjects was amplified by non-nested PCR to give five partly overlapping amplicons of ~ 1000 bp, which together covered the whole protein-coding part of the genome. For these reactions, undiluted DNA samples were used to ensure that the resulting PCR product would represent the most common sequence of the viral DNA molecules present in the sample. Before sequencing, the PCR products were purified with the PCR product purification kit (Boehringer Mannheim). The same primers were used both for the PCR and the sequencing reactions. Sequencing was done (at the Institute of Biotechnology, University of Helsinki) from both ends of the amplicons. The primers were NSofwd and NSirev, NSsfwd and NSorev, p6 and p3, p9 and rtsrev and the last pair was rt1 and VP2orev (Table 1).

■ **Phylogenetic analysis.** Phylogenetic analysis was performed on a 346 nt region corresponding to nt 2246–2789 of the viral genome. Sequence alignments were done by using CLUSTAL W version 1.75 (Thompson *et al.*, 1994) and subsequent analyses were done by using the PHYLIP package (Felsenstein, 1989).

Results

B19 DNA in tissues and serology

Synovial tissues obtained from otherwise healthy patients undergoing arthroscopic surgery for joint trauma were screened for B19 DNA by amplifying a fragment in the middle of the genome, covering the junction of the NS1 and VP genes (VP1 PCR). Blood samples taken from the same patients were studied for B19 antibodies. We had both tissue and serum from 30 patients, of which 12 had no B19 antibodies and 16 had B19

Table 2. Results from the different B19 PCR tests

The dilutions given as the VP1 PCR end-points were used subsequently for the NS1, VP2 and duplex PCRs.

Sample	Antibody status	VP1 PCR end-point	NS1 PCR	VP2 PCR	Duplex PCR
1	IgG^+ IgM^-	10	+	+	ND
2	IgG^+ IgM^-	10	+	+	+
3	IgG^+ IgM^-	100	+	+	+
4	IgG^+ IgM^-	1000	+	+	ND
		10000	−	−	ND
5	IgG^+ IgM^+	1000	+	+	+
6	IgG^+ IgM^+	1000000	+	+	+

ND, Not done.

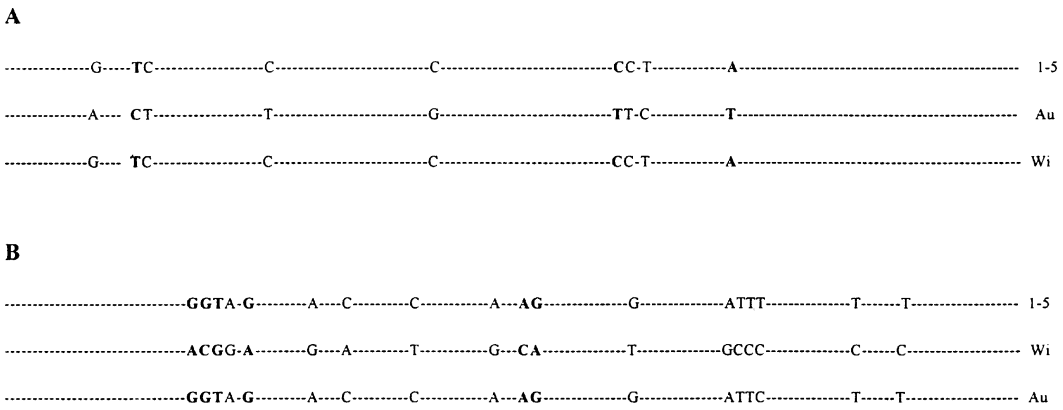

Fig. 1. Conserved nucleotide changes in synovial B19 DNA (labelled 1-5) relative to the Au sequence (A) and the Wi sequence (B). Changes occurring consistently in all five tissues studied (except nt 480, which was analysed in four tissues) are shown on the first row. Non-coding changes are marked in normal font and coding changes are in bold.

IgG but no B19 IgM, whereas two patients had both B19 IgG and IgM. On the other hand, of the 18 B19-seropositive patients, 12 (67%) had B19 DNA in synovium, whereas all the seronegative subjects were devoid of synovial B19 DNA. Thus, as in the previous study (Söderlund *et al.*, 1997), every patient PCR-positive had been infected with the virus. Of the B19 DNA-positive synovium, we chose four cases randomly for further examination representing long-term carriership of B19 DNA (positive IgG, negative IgM, 'non-acute' ETS patterns) and, for comparison, two cases with serological evidence of recent B19 infection (positive IgG, positive IgM, 'acute' ETS patterns). The six samples chosen for further study were titrated serially in 10-fold steps for VP1 PCR positivity.

NS1 and VP2 PCR

The last dilutions of DNA that gave a positive VP1 signal (end-point dilutions) were used as the template in PCR assays for the VP2 and NS1 genes. The entire protein-coding area of the B19 genome could be found in all six samples (Table 2). With five of these samples, the last dilution that was positive in VP1 PCR also gave a positive result in VP2 and NS1 PCR assays. With the one sample positive in VP1 PCR in dilutions up to 1:10000, the NS1 and VP2 regions could be detected in dilutions up to 1:1000 (Table 2).

Duplex PCR

In order to assess whether the tissues contained the entire B19 coding region and whether the DNA was intact or fragmented, both ends of the coding region were co-amplified in end-point-diluted templates. The NS1 and VP2 regions were detectable simultaneously in all four samples studied.

Sequencing

Sequencing was performed on five subjects as outlined in Methods. An almost-complete sequence was obtained for four of these samples, with just the two terminal primers and 4–38 nucleotides directly internal of them being unreadable. At approximately nt 3800 of the viral genome, i.e. near the extreme limit of sequence readability from either end of one particular amplicon, another small gap of $\leqslant 26$ nt was not readable for three of four samples. In all, $> 97\%$ of the B19 coding region of 4354 nt of the Au strain (corresponding to a coding sequence of 4362 nucleotides) was sequenced from each of the four isolates (Kati 1–Kati 4).

Both in our patients with recent infection and in our subjects with past immunity, the sequence identity relative to the Au reference was $> 99\%$. Altogether, our synovial sequences differed from either reference (Au or Wi) by 27 conserved (i.e. occurring in every subject studied) nucleotide changes. Yet, all our conserved changes relative to either reference strain were found to agree with the other reference strain. Of the nine conserved changes relative to Au, five were within the NS gene and four within the VP1/2 gene (Fig. 1). While six of these mutations were silent, three changed an amino acid; nt 692 converted threonine to isoleucine, nt 3809 converted serine to threonine and nt 3182 converted serine to proline. Conversely, our synovial sequences differed from the Wi reference in 18 conserved changes. For all 18 nucleotides, our sequences were identical to the Au reference (Fig. 1).

The Au and Wi references differ from each other by 35 nucleotides within the region sequenced here. For the eight nucleotides in addition to the 27 described above, all our sequences individually followed either the Au or the Wi reference.

We next examined our sequence data for evidence of non-conserved mutations that might inactivate the B19 proteins functionally. Throughout our samples, non-silent mutations were rare, constituting $< 25\%$ of all nucleotide differences (Table 3). Of the few that were found, none involved changes in amino acid side groups that would appear to cause gross disruption of the protein structure. In addition, no stop codons, frame-shifts, insertions or deletions were found.

Phylogenetic analysis

In an effort to look for additional evidence of genetic clustering or abnormal variation of our sequences from other B19 genotypes, we performed phylogenetic analysis using synovial DNA of two patients with recent B19 infection and of two patients with long-term B19 carriership. To make comparisons against the largest possible data set, we chose the VP1 unique region for these studies. Using our data along with 24 analogous B19 regions from GenBank, neither of the methods tried (neighbour-joining or maximum parsimony) was able to yield a phylogeny with generally well-resolved nodes. Although some sequence isolates were observed to cluster reliably, no grouping of any of our sequences with extant B19 sequences or with each other could be demonstrated. This supports our conclusion, based on individual mutations across the whole coding sequence, that no unique genotype is correlated with synoviotropism or synovial retention.

Discussion

By using three distinct (NS1, VP1, VP2) nested PCR methods, we showed that the synovial tissue, both in recently infected and previously infected subjects, contains the entire B19 coding region. The methods were optimized to give similar sensitivity, theoretically detecting a single target molecule (Söderlund *et al.*, 1997). Even if our techniques were not genuinely quantitative, their performance at limiting dilution allows for the approximation that all B19 genomic regions were present in equimolar amounts. Further support for the integrity of the B19 DNA came from duplex amplification at apparently equal efficiency, simultaneously at both ends of the viral genome, again performed at limiting template dilution.

The B19 genome has long palindromic repeats at both termini (Deiss *et al.*, 1990; Shade *et al.*, 1986; Astell & Blundell, 1989) consisting mainly of GC base pairs, which, together with their hairpin secondary structure, provide extremely difficult targets for PCR detection. We did not examine these non-coding termini. However, with an intact, full-length coding region present in synovium, viral mRNA and protein could potentially be produced; the B19 genomes could then possibly have a pathogenetic role. Recent findings suggesting such a role (Takahashi *et al.*, 1998) await confirmation.

A single point mutation in a viral coding region can alter host-cell tropism or might lead to organ-specific persistence. The host-range determinants of the non-human parvoviruses minute virus of mice (MVM) and feline and canine parvoviruses (FPV; CPV) have been characterized in detail. Just two amino acids of the capsid region determine the lymphoid-cell tropism of the MVM variant MVMi and the fibroblast tropism of MVMp (Ball-Goodrich & Tattersall, 1992). Likewise, a change of only a few amino acids yields canine tropism for FPV (Chang *et al.*, 1992). However, with the exception of a recently discovered variant strain, V9 (Nguyen *et al.*, 1998), the human B19 viruses from blood or bone marrow characterized until now have differed by conspicuously little in their sequences. Neither have disease-specific variants been reported. Similarly, the B19 coding sequences that we detected in synovium resembled closely those of two reference strains from blood, Au (Shade *et al.*, 1986) and Wi (Blundell *et al.*, 1987). This implies that the tropism for and genome persistence in synovial tissue are not related to specific mutations or strain variants. Such a conclusion is supported further by phylogenetic analysis of the VP1 unique region, in which our sequences showed no clustering apart from the known blood-derived sequences. Considering the immunological importance of the VP1 unique region, our data suggest further that neither synoviotropism nor persistence of the B19 virus is due to sequence-based evasion of host immunity. Interestingly, this same capsid protein region maintains IgG subclass 4 reactivity, a marker of chronic or recurrent antigenic stimulus (Franssila *et al.*, 1996).

Whereas in permissive cells, the VP transcripts outnumber the NS1 transcripts, the latter predominate in some non-permissive cell types (Liu *et al.*, 1992; Leruez *et al.*, 1994). As NS1 regulates B19 gene expression via the p6 promoter (Doerig *et al.*, 1990), mutations in NS1 affecting its trans-activation capacity could have pleiotropic effects on the pathogenicity of B19 infection, for example via modulation of its cytotoxicity. However, no conserved mutations pointing to this mechanism in synovial persistence were observed. With B19 detected in blood (presumably reflecting ongoing replication in the marrow), sequences of the NS1 region have been reported to be somewhat more variable during long-term persistence than in acute infection (Hemauer *et al.*, 1996). In our synovial samples, the recently and remotely infected individuals also resembled each other in extent of variability, although none of the sequences were identical. With these findings, it is tempting to speculate that the mechanisms of B19 persistence in bone marrow and synovium are different, so that only the former depends on continuous DNA replication. A larger sample is required for verification of this hypothesis.

Taken together, our data show that neither short-term synoviotropism nor long-term synovial persistence of parvovirus B19 require DNA sequence modifications. The single-stranded DNA would appear to enter the joint tissue at its full size and to be retained there for years or even decades (Saal *et al.*, 1992; Kerr *et al.*, 1995; Söderlund *et al.*, 1997; Cassinotti *et al.*, 1997) without fragmentation. For such an innocuous role, macrophages or other actively hydrolytic cells would seem an unlikely candidate.

Synovial lining cells can be roughly divided into three populations according to morphology and surface antigen expression (Barland *et al.*, 1962; Burmester *et al.*, 1983). Type A lining cells express antigens of the monocyte lineage and conform to the criteria of mature macrophages that are relatively long lived and originate from the bone marrow

Table 3. Codons of synovial B19 DNA that differ from the Au reference

Entries in bold represent conserved changes relative to the Au sequence.

Position	Sample 1 (acute)	Sample 2 (persistence)	Sample 3 (persistence)	Sample 4 (acute)	Sample 5 (persistence)	Amino acid change
480	**CTA → CTG**	**CTA → CTG**	**CTA → CTG**	**CTA → CTG**		–
544	CTA → TTA					–
640			TTT → CTT		TTT → CTT	F → L
660		AAA → AAG				–
692	**ACT → ATT**	**ACT → ATT**	**ACT → ATT**	**ACT → ATT**	**ACT → ATT**	T → I
723	**CTT → CTC**	**CTT → CTC**	**CTT → CTC**	**CTT → CTC**	**CTT → CTC**	–
726			ACA → ACT		ACA → ACT	–
783				GTG → GTA		–
789		CTA → CTG				–
811	ACA → TCA					T → S
957	TGC → TGT					–
1004			AGT → ACT		AGT → ACT	S → T
1024		GGC → AGC				G → S
1059	AAG → AAA					–
1062	GGA → GGC					–
1074				AGC → AGT		–
1112		AAC → ACC				N → T
1119				GTG → GTA		–
1125			ACA → ACT		ACA → ACT	–
1227		TAT → TAC				–
1254				AGC → AGT		–
1278				TTT → TTG		F → L
1344			CCC → CCT		CCC → CCT	–
1392	**GGT → GGC**	**GGT → GGC**	**GGT → GGC**	**GGT → GGC**	**GGT → GGC**	–
1395			AAG → AAA		AAG → AAA	–
1530		GGA → GGG	GGA → GGG		GGA → GGG	–
1569		TCT → TCA				–
1960	ATC → GTC					I → V
2011			TTT → CTT		TTT → CTT	F → L
2096	TTT → TCT			TTT → TCT		F → S
2241			GTG → GTA	GTG → GTA	GTG → GTA	–
2268	**GGG → GGC**	**GGG → GGC**	**GGG → GGC**	**GGG → GGC**	**GGG → GGC**	–
2331	GAA → GAG					–
2352		CGA → CGG	CGA → CGG		CGA → CGG	–
2394			CTA → CTG			–
2400		TGC → TGT				–
2453		AAA → GAA		AAA → GAA		K → E
2539			GGA → GGT			–
2581			AAT → AAC			–
2599				CCC → CCT		–
2654			AAA → GAA			K → E
2762	AAT → GAT			AAT → GAT		N → D
2828/2829				AAC → GCC		N → A
2879				GTT → ATT		V → I
2919				CAA → CTA		Q → L
2962		GTA → GTG				–
2977					CTT → CTC	–
3182	**TCT → CCT**	**TCT → CCT**	**TCT → CCT**	**TCT → CCT**	**TCT → CCT**	S → P
3187	GTC → GTT		GTC → GTT		GTC → GTT	–
3214			ACT → ACA		ACT → ACA	–
3223	**GCT → GCC**	**GCT → GCC**	**GCT → GCC**	**GCT → GCC**	**GCT → GCC**	–
3271				GAC → GAT		–
3289			AAG → AAA		AAG → AAA	–
3307	GCG → GCA	GCG → GCA		GCG → GCA		–
3313	AGT → AGC		AGC → AGT		AGC → AGT	–

Table 3 (*cont.*)

Position	Sample 1 (acute)	Sample 2 (persistence)	Sample 3 (persistence)	Sample 4 (acute)	Sample 5 (persistence)	Amino acid change
3355	**ATC → ATT**	**ATC → ATT**	**ATC → ATT**	**ATC → ATT**	**ATC → ATT**	–
3358				AGT → AGC		–
3361	CCC → CCT					–
3412	AAT → AAA					N → K
3463			AGT → AGC		AGT → AGC	–
3469			GCT → GCC		GCT → GCC	–
3541	GTA → GTG			GTA → GTG		–
3547		GTT → GTC				–
3583		GTA → GTG				–
3625	CAG → CAA			CAG → CAA		–
3628		GAT → GAC				–
3721	GGA → GGT					–
3809	**TCT → ACT**	**TCT → ACT**	**TCT → ACT**	**TCT → ACT**	**TCT → ACT**	S → T
3859		TGC → TGT				–
3943		TTT → TTC				–
3988	TTC → TTT					–
4093	AAC → AAT					–
4096			ACT → ACC			–
4106/4108		TTA → CTC				–
4132	CCA → CCG			CCA → CCG		–
4162			GTC → GTT			–
4192				CAG → CAA		–
4240	GGT → GGG		GGT → GGC			–
4261		GAA → GAG				–
4270		AAA → AAC				K → N
4300			TAC → TAT			–
4423	TTA → TTG					–
4570		GCC → GCT	GCC → GCT			–
4591	ACT → ACC			ACT → ACC		–
4609	GGG → GGT			GGG → GGT		–
4624	ACG → ACA	ACG → ACA				–
4642			CAA → CAG			–
4657			CCC → CCA			–
4708		ACA → ACG				–

(Edwards & Willoughby, 1982), where B19 replicates. Type B synoviocytes are fibroblastoid in morphology and express fibroblast-associated antigens (Burmester *et al.*, 1983). However, they differ from fibroblasts of other organs in a number of respects, e.g. in certain diseases and *in vitro* conditions they are rich in lysosomes (Fraser *et al.*, 1979), pointing to active extracellular uptake. The third, minor population includes cells with dendritic appearance and other characteristics of antigen presentation and/or storage (Wilkinson *et al.*, 1990). Our data would best fit retention of B19 virus genomes in synoviocytes of the third category, which in turn would underline the importance of synovium in immunological processes, possibly as a site for maintenance of memory. Indeed, identification of the cell type(s) hosting B19 virus persistence in the joint tissue and the transcriptional and replicative functionality of the viral genomes therein will be exciting subjects for future study.

We thank Robert von Essen and Alex Plyusnin for discussions and Lea Hedman for technical assistance. This work was supported by grants from the Helsinki University Central Hospital Research and Education Fund, the Finnish Technology Advancement Fund, the Finnish Science Academy and the Centre for International Mobility.

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Received 25 November 1999; Accepted 23 December 1999