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Characterization of three co-circulating genotypes of the small hydrophobic protein gene of mumps virus

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Eighteen virus isolates and 22 serum samples collected between 1971 and 1997 from patients with mumps were genotyped by PCR with specific primer pairs for the A, C and D genotypes of the small hydrophobic (SH) protein gene. All serum samples were subjected to nucleotide sequence analysis of the gene, and the deduced 57-amino-acid sequences were aligned with previously published sequences from the USA, Canada, Portugal, the UK, France, Germany, Switzerland, Denmark, Sweden, Russia, China and Japan. The existence of six genotypes of the SH protein gene, named A to F, was confirmed. In the Stockholm area, co-circulation of genotypes A, C and D at different times was found. There was a striking difference in genotype between the virus isolates and the serum samples. The 18 virus isolates represented genotypes C and D, whereas the 22 serum samples contained genotype A. In most cases, the amino acid sequences of the 22 genotype A specimens were identical to the previously described SBL-1 strain of genotype A. Genotypes C and D were always associated with meningitis, and in some cases parotitis, whereas infection with genotype A most often resulted in parotitis and seldom in meningitis.

Introduction

Mumps virus belongs to the family *Paramyxoviridae*, genus *Rubulavirus* (Rima *et al.*, 1995). The single-stranded mumps virus genomic RNA contains seven genes, in the following order on the genome map: the nucleocapsid (N), phospho (P), membrane (M), fusion (F), small hydrophobic (SH), haemagglutinin–neuraminidase (HN) and large (L) protein genes (Elango *et al.*, 1988; Elliott *et al.*, 1989). The SH gene of mumps virus encodes a protein of 57 amino acids (Takeuchi *et al.*, 1996). Reduced or absent expression of the SH gene, with a concomitant reduction of SH protein, has been described for certain mumps virus strains (Afzal *et al.*, 1990; Takeuchi *et al.*, 1991, 1996). Lack of expression of the SH protein does not affect virus replication *in vitro*, but may modify virus pathogenesis *in vivo* (Takeuchi *et al.*, 1996). Comparisons of the nucleotide sequences of the SH gene from different mumps virus isolates from around the world have shown the existence of six genotypes, which have been named A to F (Takeuchi *et al.*, 1991; Yeo *et al.*, 1993; Künkel *et al.*, 1994, 1995; Afzal *et al.*, 1997*a*, *b*; Örvell *et al.*, 1997*a*; Saito *et al.*, 1998; Wu *et al.*, 1998). The possible existence of a seventh genotype has been reported by Ströhle *et al.* (1996). In Europe, the A, C, D and E genotypes have been identified (Yeo *et al.*, 1993; Künkel *et al.*, 1994, 1995; Örvell *et al.*, 1997*a*; Afzal *et al.*, 1997*a*, *b*). In Japan, the B and D genotypes have been found (Takeuchi *et al.*, 1991; Yeo *et al.*, 1993; Afzal *et al.*, 1997*a*; Örvell *et al.*, 1997*a*; Saito *et al.*, 1998). A recent mumps outbreak in Japan with a high incidence of aseptic meningitis was found to have been caused by genotype D (Saito *et al.*, 1996, 1998). The most recently discovered genotype, F, was isolated in China in 1995 and 1996 (Wu *et al.*, 1998).

The occurrence of sporadic mumps outbreaks is a well-known phenomenon, even in populations with high vaccine coverage (Wharton *et al.*, 1988; Hersh *et al.*, 1991; Briss *et al.*, 1994; Künkel *et al.*, 1994, 1995; Germann *et al.*, 1996; Ströhle *et al.*, 1996; Afzal *et al.*, 1997*b*). In the epidemics in Switzerland in 1992–1993 and 1995 and in Portugal in 1996, the population was vaccinated with a heterologous genotype (Künkel *et al.*, 1994; Ströhle *et al.*, 1996; Afzal *et al.*, 1997*b*). Taken together, these reports indicate that the vaccine may not be effective against heterologous virus genotypes. Antigenic diversity between mumps virus strains identified by monoclonal antibodies has been reported for the P, N, F and HN proteins (Server *et al.*, 1982; Örvell, 1984; Rydbeck *et al.*, 1986; Yates *et al.*, 1996; Örvell *et al.*, 1997*b*). Some monoclonal antibodies with high neutralizing activity directed against the HN protein

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Table 1. Mumps virus samples

Samples are arranged by year of collection. NK, Not known; E, encephalitis; M, meningitis; P, parotitis.

Sample	Year	Genotype	Material	Age of patient (years)	Clinical symptoms
F 27905 V7	1971	D	CSF	10	M, P
F 27813 V8	1971	D	CSF	24	M, P
F 30888 V9	1972	D	CSF	14	M
F 31381 V13	1972	D	CSF	5	M
F 33596 V12	1973	D	CSF	4	M, P
F 39404 V15	1975	D	CSF	8	M, E, P
F 39971 V16	1975	D	CSF	3	M
F 39953 V17	1975	D	CSF	8	M, P
F 39676 V18	1975	D	CSF	10	M, P
F 49685 V19	1978	D	CSF	7	NK
F 49745 V20	1978	D	CSF	16	NK
F 55640 V21	1979	D	Urine	15	M, P
F 55634 V22	1979	D	CSF	12	M, P
F 55622 V23	1979	D	CSF	1	NK
F 58013 V24	1980	D	CSF	7	NK
SD 1320	1980	A	Serum	24	M, P
SD 1589	1980	A	Serum	9	Fever, vomiting
SD 1738	1980	A	Serum	15	NK
SD 1939	1980	A	Serum	10	NK
SD 2438	1980	A	Serum	34	M, P
SD 2667	1980	A	Serum	4	P
SD 2793	1980	A	Serum	31	E
SD 3029	1980	A	Serum	37	P
SD 3739	1980	A	Serum	3	P
SD 4019	1980	A	Serum	27	M
F 58287 V26	1980	D	CSF	12	NK
F 72075 V27*	1983	C	CSF	4	M
F 72341 V28*	1983	D	CSF	6	M, P
F 74584 V29*	1983	C	CSF	5	M
F 75987 V30	1984	C	CSF	31	M
F 75526 V31*	1984	C	CSF	12	M
F 75653 V32	1984	C	CSF	NK	NK
F 77139 V33*	1984	D	Urine	17	M
F 80289 V34*	1984	C	CSF	NK	NK
F 81132 V35*	1985	D	CSF	6	M
VS93 4098*	1993	A	Serum	6	P†
VS93 6091	1993	A	Serum	5	P
VS94 4482	1994	A	Serum	4	P†
VS94 5299	1994	A	Serum	29	P
VS95 666	1995	A	Serum	4	M, E
VS95 2309	1995	A	Serum	4	P†
VS96 2921	1996	A	Serum	27	P
VS96 3592	1996	A	Serum	51	Orchitis
VS96 4922	1996	A	Serum	63	P
VS96 5262	1996	A	Serum	34	P
VS97 2245	1997	A	Serum	33	P
VS97 3262	1997	A	Serum	8	P
MV97 708	1997	D	CSF	16	M

* These samples have been genotyped previously (Örvell *et al.*, 1997*a*).
† These patients were vaccinated at about 2 years of age.

of the SBL-1 strain (genotype A) and the Urabe strain (genotype B) have been found to react with homologous but not heterologous virus strains (Örvell, 1984; Yates *et al*., 1996; Örvell *et al*., 1997*b*). Recent studies have demonstrated that epitopes which give rise to type-specific neutralizing antibodies are well conserved over long periods of time (Yates *et al*., 1996; Örvell *et al*., 1997*b*). Genotype A appears to show the most pronounced difference in neutralizing epitopes, whereas genotypes B, C and D share a number of neutralizing epitopes (Yates *et al*., 1996; Örvell *et al*., 1997*b*).

The aim of the present study was to genotype and sequence the SH gene of viruses circulating in the Stockholm area over a long time-period, and to compare the deduced amino acid sequences of these viruses to the vaccine virus in use in Sweden, and also to other sequenced virus genotypes from different parts of the world. We were also interested in developing an easy and rapid method to genotype different mumps virus strains, by use of the PCR with specific primers for each genotype. Attempts were also made to compare the clinical symptoms of patients, to see if the genotypes exhibited a different pathogenetic behaviour *in vivo*.

Methods

■ **Materials for analysis.** Sixteen mumps virus strains isolated from cerebrospinal fluid (CSF) and two virus strains isolated from urine of patients with mumps between 1971 and 1985 were used in the present investigation. The material has been partly characterized previously, by immunofluorescence analysis with monoclonal antibodies (Örvell *et al*., 1997*b*; Table 1). It was shown by this method that none of these 18 virus isolates reacted with type-specific monoclonal antibodies directed against genotype A. Twenty-two serum samples and one sample from the CSF from patients with mumps were used directly for amplification and genotyping by PCR and nucleotide sequencing (Table 1). Background clinical information was collected on the cases. Age of patients, symptoms, vaccination history and IgG and IgM antibody titres of serum samples were recorded, where this information was available.

■ **Genotyping by PCR.** Genotyping by PCR was performed only on materials which were shown first to be successfully amplified in a nested PCR with primers common to all known genotypes of the SH protein gene, as described previously (Örvell *et al*., 1997*a*). Isolation of mumps virus RNA from the different samples was performed essentially as described for hepatitis C virus by Yun *et al*. (1993). The outer primers, BJSHPR3 and BJSHPR4, amplified a 430 bp fragment flanking the SH gene (Örvell *et al*., 1997*a*). The inner primers in the nested PCR reaction, specific for the A, C and D genotypes, are presented in Table 2. The binding sites of the different primer pairs on the virus genome were numbered according to Elliott *et al*. (1989). The length of the final amplified product was 156, 128 and 105 bp for genotypes A, C and D, respectively. The nested PCR consisted of an initial cycle of denaturation at 95 °C for 2 min, annealing at 63 °C (genotype A and C) or 53 °C (genotype D) for 30 s and elongation at 72 °C for 1 min. The first cycle was followed by 34 cycles of denaturation at 95 °C, annealing as above for 30 s and extension at 72 °C for 1 min. After the nested PCR, the sizes of the amplified fragments were examined by gel electrophoresis, with a *Hae*III digest of ϕX-174 RF DNA included as a size marker.

■ **Nucleotide sequencing.** For nucleotide sequencing of the SH gene, a 415 bp fragment was amplified by PCR as described previously (Örvell *et al*., 1997*a*). For each fragment, one of the inner primers (BJSHPR2) was 5′-biotinylated and the other (BJSHPR1) was coupled at the 5′ end to a universal M13 primer sequence (5′ CGACGTTGTAAAACGGCCAGT 3′). Direct DNA sequencing was performed as described previously (Örvell *et al*., 1997*b*). For some serum samples, nucleotide sequencing was performed after a nested PCR amplification of a fragment of the HN gene with the following inner primers: sense primer 5′ CCAATATCTCAGTGATGGCCTGAAT 3′, nucleotide positions 773–797 (SBL-1 HN gene, GenBank accession no. M55065); antisense primer 5′ CCCACTCCTGGCACCAAAGT 3′, positions 984–1003 (Kövamees *et al*., 1989).

■ **ELISA.** Titres of IgG and IgM antibodies in patients' sera were determined by ELISA according to accredited test procedures at the Department of Clinical Virology, Huddinge University Hospital. A preparation of purified glycoproteins of the SBL-1 strain was used as the antigen for determination of IgG titres (Örvell, 1978; Linde *et al*., 1987). For IgM determination, an IgM capture procedure was used. The ELISA plates were coated with goat anti-human IgM antibodies. After the addition of 0·1 ml aliquots of different dilutions of the serum samples to

Table 2. Oligonucleotide primers used in genotyping

Positions of primer binding sites are given according to the numbering of nucleotides of Elliott *et al*. (1989). Degenerate bases within primers are indicated by R (representing A or G), Y (C or T) and M (A or C). NA, Not applicable.

Primer	Binding site	Sequence	Product (bp)
BJSHPR3	NA	5′ CGATGATCTCATCAGGTACT	430
BJSHPR4	NA	5′ TCCTAAGTCTGTTCTGGCTT	
MUMSHA1	3178–3202	5′ AACTCTGTATGTCTGGACTATATTG	156
MUMSHA2	3314–3333	5′ CTTGTTCTAGCGTGACGGAT	
MUMSHC1	3178–3197	5′ AACTTTGTATGTCTGGGTCG	128
MUMSHC2	3285–3305	5′ GTCCTAACTGGAGATCTTTCT	
MUMSHD1	3229–3247	5′ RCATGMAGYAYTGYACCAA	105
MUMSHD2	3314–3333	5′ CTTGTTYACRCATRAGRGAY	

each well, the ELISA plates were incubated at 37 °C for 2 h. After washing of the plates, 0·1 ml purified virions of the SBL-1 strain of mumps virus, containing 1 μg viral protein, was added to each well. After a further incubation at 37 °C for 2 h, and subsequent washing, a predetermined dilution of a peroxidase-labelled monoclonal antibody directed against the NP protein was added (Örvell, 1984). After a final incubation for 1 h, and subsequent washing, 5-aminosalicylate was added and, after incubation at room temperature for 30 min, the absorbance at 549 nm was determined in a spectrophotometer.

Results

Genotyping of mumps virus by PCR

Both virus isolates and serum samples were used for genotyping by PCR. The results from genotyping by PCR correlated with results obtained by nucleotide sequencing. The bands of the amplified products of genotype A (156 bp), genotype C (128 bp) and genotype D (105 bp) showed the expected sizes in relation to the markers (Fig. 1). The primer pair MUMSHA1/MUMSHA2 did not cross-react with either genotype C or D at any annealing temperature tested (not shown); the annealing temperature chosen was 63 °C. The primer pair MUMSHD1/MUMSHD2 showed some cross-reaction with genotype C at low annealing temperatures (45 °C), but this was easily rectified by raising the annealing temperature to 53 °C. The primer pair MUMSHC1/MUMSHC2 cross-reacted with genotype D to some extent. In order to reduce this cross-reactivity, the annealing temperature was raised to 63 °C and the amount of each primer used was decreased from 20 pmol to 10 pmol. Under these conditions, cross-reactivity decreased, but did not disappear altogether. In cases where cross-reactions occurred between the C and D genotypes, tenfold titration of the first amplified product was performed before the nested PCR. Under these conditions, less dilution was necessary to prevent amplification of product by the heterologous primer pair than by the homologous primers.

Mumps virus genotypes in different clinical materials

Eighteen virus isolates collected between 1971 and 1985 were genotyped (Table 1). Sixteen isolates collected between 1971 and 1980 contained genotype D. For samples from 1983 to 1985, both genotypes C and D were identified in the virus isolates. Since 1985, no mumps virus isolates have been recovered in our laboratory. All the virus isolates except two (F55640 V21 and F77139 V33) were recovered from the CSF. From this group, clinical information was available for 18 patients. All 18 patients exhibited clinical signs of meningitis, and meningitis was the only reported clinical sign in nine of the patients (Tables 1 and 3). The remaining nine patients also exhibited parotitis. For the four patients from whom genotype C was isolated, meningitis was the only recorded symptom (Table 3).

A CSF sample (MV97 708) taken recently from an unvaccinated patient with meningitis was also demonstrated to contain genotype D. This virus was genotyped directly from CSF, as attempts to isolate the virus in tissue culture were not successful.

Serum samples were collected in 1980 and between 1993 and 1997 (Table 1). The serum samples from 1980 consisted of a serum panel of acute and convalescent serum from each patient. The acute serum samples, which were used for genotyping, were taken 3–10 days after the onset of disease. All acute samples contained high IgM antibody titres and low or absent IgG titres, characteristic of primary infection (not shown). Ten of the serum samples collected between 1993 and 1997 contained IgM antibodies. Two serum samples showed serological evidence of reinfection, with the absence of IgM and the presence of IgG antibodies in the acute serum (serum samples VS94 5299 and VS97 2245). All 22 serum samples contained genotype A. Clinical information was available from 20 of the patients. Thirteen of the 20 patients had parotitis as the only reported clinical sign (Tables 1 and 3). Four of the

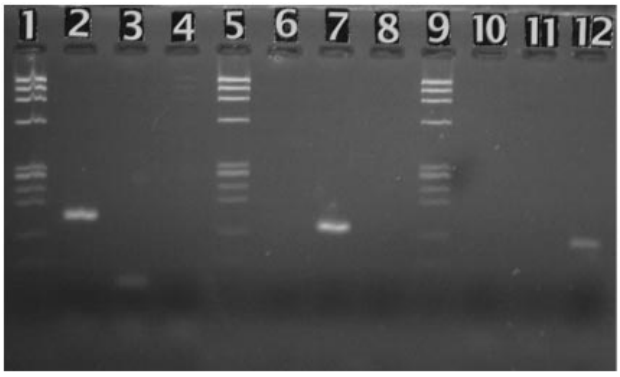

Fig. 1. Genotyping of mumps virus isolates by PCR. DNA from genotypes A (lanes 2, 6 and 10), C (lanes 3, 7 and 11) and D (lanes 4, 8 and 12) was included on the gel. DNA from the different genotypes had been subjected to a nested PCR with primers specific for genotypes A (lanes 2–4), C (lanes 6–8) or D (lanes 10–12). Molecular mass markers were run in lanes 1, 5 and 9.

Table 3. Relationship between mumps virus genotypes and clinical symptoms

Only patients for whom information on clinical symptoms was available are included.

Genotype	No. of cases	No. of patients with: Parotitis only	Meningitis and parotitis	Meningitis only
A	20	13	2	2
C	4	0	0	4
D	14	0	9	5

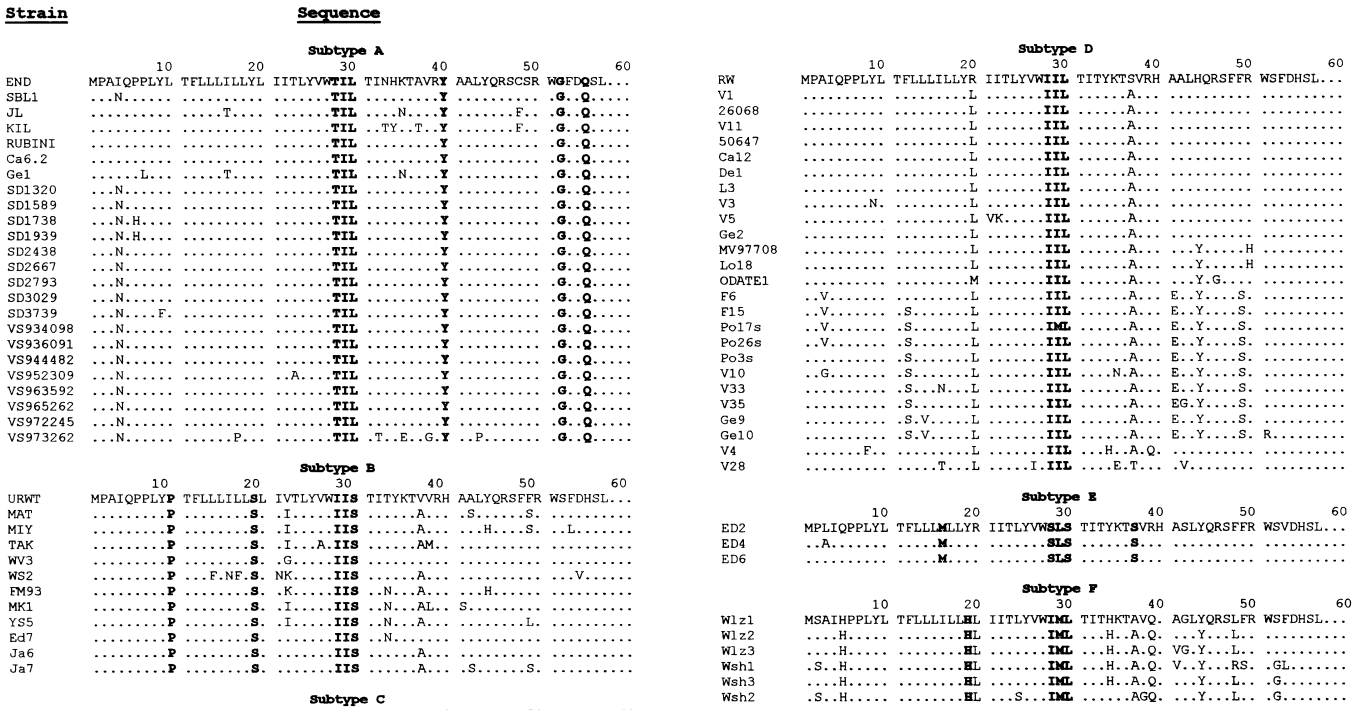

Fig. 2. Deduced amino acid sequences of the SH protein gene. Sequences were obtained from the following: Elango *et al.* (1989), Takeuchi *et al.* (1991), Yeo *et al.* (1993), Künkel *et al.* (1994, 1995), Afzal *et al.* (1993, 1997 *a*, *b*), Örvell *et al.* (1997*a*), Saito *et al.* (1998), Wu *et al.* (1998), the present study and GenBank.

patients had meningitis. Samples for virus isolation were taken from the CSF of five of these patients (SD 1589, SD 1939, SD 2438, SD 4019 and VS95 666), and mumps virus was isolated from two cases (SD 1589 and SD 1939). The geometric mean age of patients with genotype C or D infection was 10 years and the geometric mean age of patients with genotype A infection was 21 years.

Nucleotide sequencing and deduced amino acid sequence of the SH gene

The 22 serum samples that were shown by PCR to contain genotype A were subjected to nucleotide sequencing of the SH gene. The deduced amino acid sequences of 17 samples are shown in Fig. 2. In most cases, the amino acid sequences were identical to the SBL-1 strain, which was isolated more than 25 years ago (Rydbeck *et al.*, 1986). All samples contained asparagine in the fourth position, which distinguished the virus strains in Stockholm from other known genotype A strains.

It is known that the SBL-1 strain contains two unique amino acids, at positions 265 and 266 of the HN protein, that differ from other known genotype A strains (Waxham *et al.*, 1988; Kövamees *et al.*, 1989; Elliott *et al.*, 1989; Afzal *et al.*, 1993; Yates *et al.*, 1996). This region of the gene was sequenced for ten of the samples, and the amino acid sequences are shown in Fig. 3. The amino acid sequences of all ten samples were identical to the SBL-1 strain. They contained alanine and asparagine at positions 265 and 266 of the HN protein, respectively, which distinguished them from other genotype A strains. The CSF sample of genotype D (MV97 708) from a patient with meningitis exhibited an amino acid sequence

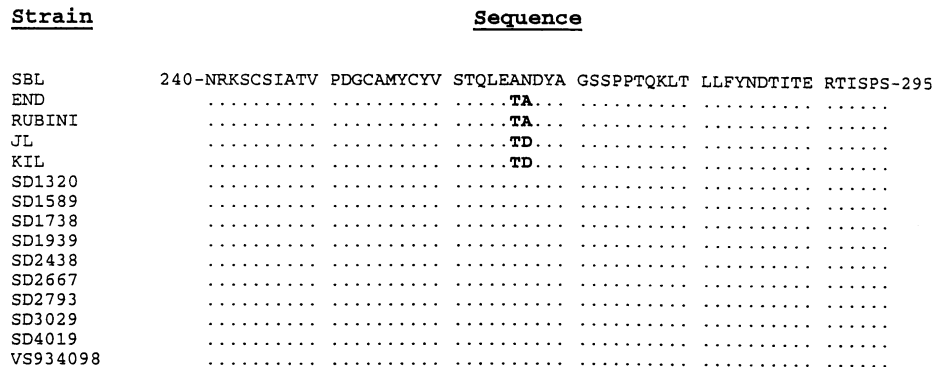

Fig. 3. Deduced amino acid sequence of positions 240–295 of the HN protein from ten genotype A virus samples from Stockholm. The sequences were compared to the SBL-1 strain and the published sequences of four other genotype A strains.

which was identical to the strain Lo18, isolated in London in 1991 (Fig. 2; Afzal *et al.*, 1997*a*).

Comparison of the deduced amino acid sequences of SH protein genes from the six existing genotypes

The amino acid sequences deduced from a large number of SH genes of mumps virus from different parts of the world were compared (Fig. 2). The different genotypes show variable degrees of conservation when members are compared within groups. Genotype A shows a high degree of conservation over long periods of time. Strains from Stockholm were noticeably different from other genotype A strains at the fourth amino acid position. Also, genotype C showed a high degree of conservation. Conservation over time is less well recorded for genotype C, as the oldest virus isolates identified so far are from 1983 (Örvell *et al.*, 1997*a*). The genotype B virus strains were isolated between 1970 and 1995; a low degree of heterogeneity was found in this group.

The most variable genotype appears to be D. At least two different groups, on the basis of amino acid sequences, appear to exist within this genotype. One group consists of strains L3, RW, V1, V3, V5, 26068, V11, Cal2, 50647, De1 and Ge2, isolated in Russia, the USA, Stockholm, Denmark and Germany between 1953 and 1987. The second group contains strains V10, V33, V35, Ge9, Ge10, F6, F15, Po17s, Po26s and Po3s, isolated in Stockholm, Germany, France and Portugal between 1972 and 1996. Strains Wlz1 to Wlz3 and Wsh1 to Wsh3, isolated in China in 1995 and 1996, have been shown to form a new genotype, F, by phylogeny analysis using nucleotide sequences (Wu *et al.*, 1998). The newly defined genotype F contains the amino acid triplet IML at positions 28–30 (Wu *et al.*, 1998).

Discussion

In a previous study, 17 virus isolates, collected between 1970 and 1985 from the CSF of patients with mumps, were genotyped by nucleotide sequencing (Örvell *et al.*, 1997*a*, *b*). In the present study, a rapid and easy test for genotyping was developed by the use of PCR with genotype-specific primers. The method may be useful for genotype screening of large numbers of specimens, but to obtain more detailed information it would be necessary to perform nucleotide sequence analysis of the SH protein gene. By the use of the two methods, 33 virus isolates collected from CSF and two isolates from urine have been genotyped. A pattern is emerging from this work. Twenty-six isolates from 1970 to 1980 contained genotype D. Nine virus isolates from 1983 to 1985 were genotyped; six of them were from genotype C and three were from genotype D. Since 1985, no virus isolation in our laboratory has been positive for mumps virus. There may be several reasons for this; the most plausible is that the occurrence of serious aseptic meningitis caused by mumps virus has drastically diminished in Stockholm. This interpretation is supported by the fact that in recent years mumps virus infection has often been diagnosed in connection with swelling of the parotid glands, but meningitis as a clinical symptom has very seldom been reported in these cases.

The absence of genotype A in the 35 virus isolates led to the conclusion that genotype A was very rare or absent in Stockholm during this time-period. Therefore, it was interesting to see whether genotype A could be found in other clinical materials from patients with mumps, such as serum. Twenty-two serum samples were investigated, 10 from 1980 and 12 from between 1993 and 1997; all contained genotype A. The 22 genotype A samples were noticeably similar in their amino acid sequences to the SH protein of the SBL-1 strain, which was isolated in Stockholm more than 25 years ago. Therefore, it is probable that this local strain has been circulating in this geographical area since this time, in contrast to genotypes C and D, which have diminished or disappeared. It is not known why genotypes C and D have diminished in Stockholm; genotype C is still circulating in other countries in Europe, and since 1985 genotype D has been isolated in France (1989) and in Portugal (1996) (Afzal *et al.*, 1997*a*, *b*). In this study, only one sample taken since 1985 was found to contain genotype D; this sample was collected in 1997 from a patient with

aseptic meningitis. The patient was a Swedish girl studying in England, where she had been staying at the time of her exposure to mumps virus (clinical information from Lars Mattson, Department of Infectious Diseases, Huddinge University Hospital).

Comparison of the available sequences reported in the literature confirmed the existence of six genotypes, named A to F. Characteristic amino acid triplets at positions 28 to 30 have been reported to be specific for each genotype (Örvell *et al*., 1997*a*). A larger amount of material was compared in the present study; this comparison shows that this amino acid triplet is not always useful for grouping of strains, e.g. strain YLB92 and Do9 of genotype C and strain Po17s of genotype D. The former strains contained the sequences VLS and VIS, respectively, rather than VVS, and the latter strain had the sequence IML instead of IIL, at these amino acid positions. Besides the six described genotypes, a possible seventh genotype has been reported by Ströhle *et al*. (1996). This strain (YLB 95), which was isolated in Switzerland in 1995, shows a remarkable similarity to a Canadian isolate (Cal.3) that was isolated in 1987 and the Bedford 1 strain, isolated in Great Britain in 1989 (Afzal *et al*., 1997*a*). All three strains have proline rather than serine in position 56, which is unique compared to SH sequences from other virus strains.

Genotype D is the most heterogeneous. At least two different groups were observed within this genotype, and a relationship with the time of isolation appears to exist between these two groups. The ODATE strain of genotype D has been reported to be associated with high neurovirulence (Saito *et al*., 1998). An interesting question is whether a connection exists between the appearance of different groups within the D genotype and changes in virus pathogenesis *in vivo*.

Vaccination against mumps in Sweden was introduced, on a small scale for selected individuals, in the late 1960s. General vaccination, with a combination vaccine against MMR (measles, mumps and rubella), was introduced in 1982 (Broliden *et al*., 1998). The vaccine contains the mumps virus strain Jeryl Lynn (genotype A). In the material examined, only samples collected from young children between 1993 and 1997 could be expected to come from vaccinated individuals. Of these young children, three had been vaccinated, two were not vaccinated and the vaccination history of the remaining two was not known. The three children that had been vaccinated exhibited a serological response of IgM antibodies characteristic of a primary infection. In these three cases, the vaccine had not protected against mumps. It is not known whether this was due to vaccine failure or waning immunity with time after vaccination. In the material collected between 1993 and 1997, two patients exhibited a serological response characteristic of re-infection.

Virus isolates from CSF samples from 32 patients with meningitis, taken between 1970 and 1985, contained genotypes C or D. No isolate belonging to genotype A was found in this material. In contrast to these findings, 10 serum samples collected in 1980 and 12 serum samples from between 1993 and 1997 contained genotype A. The latter patients most often had symptoms of parotitis, but meningitis was seldom reported. Although a definitive study cannot be undertaken due to the lack of genotypes C and D in Sweden, the results of the present study favour the conclusion that genotypes C and D cause meningitis in a higher percentage of cases than genotype A. This is supported by the fact that between 1993 and 1997, when genotype A was dominant, parotitis was almost always reported in connection with mumps but meningitis was rare. However, studies will have to be performed in countries with co-circulating genotypes A and C/D in order to confirm this conclusion. Although genotype A appears to exhibit lower neurovirulence than genotypes C and D in the present study, it can cause meningitis in some cases. Meningitis was reported in four cases of infection with genotype A, and mumps virus has been isolated from the CSF in two cases (SD1589 and SD1939). Also, in another study, it has been reported that genotype A was isolated from patients with meningitis (Künkel *et al*., 1995). In three cases described by Künkel *et al*. (1995), viruses were isolated from the throat, saliva and urine. The findings of the present study support the hypothesis of Takeuchi *et al*. (1996), that different genotypes of mumps may exhibit different virus pathogenesis *in vivo*. Defective expression of the SH protein of certain genotypes of mumps virus may explain this phenomenon (Takeuchi *et al*., 1996). In previous studies, it has been demonstrated that members of genotypes A and B show defective expression of the SH gene, with a concomitant reduction of SH protein (Afzal *et al*., 1990; Takeuchi *et al*., 1991, 1996). Further support for the existence of increased neuropathogenicity of genotype D, as described in the present study, comes from a recent study by Saito *et al*. (1998), who found that an epidemic in Japan with high incidence of meningitis was caused by the ODATE strain, belonging to genotype D. Studies in countries with co-circulating genotypes will be necessary in order to establish firmly the existence of a difference in biological behaviour *in vivo*.

A mechanism to explain the defective expression of the SH protein on a molecular basis has been proposed by Takeuchi *et al*. (1996). The same point mutation in the putative polyadenylation signal of the F gene, upstream of the SH gene, was identified in the Enders and Rubini strains and in the field isolate CH5/87, belonging to genotype A (Künkel *et al*., 1995; Takeuchi *et al*., 1996). However, this mutation was not found in the 22 genotype A strains studied in the present investigation (unpublished data). Therefore, some other factor may account for the reduced neurovirulence of the genotype A strains described in the present study.

In recent studies, it has been shown that type-specific neutralizing antibodies exist against the different mumps genotypes (Yates *et al*., 1996; Örvell *et al*., 1997*b*). Neutralizing antibodies produced in animals and directed against genotype A did not neutralize genotypes C and D effectively. In view of

the finding that genotypes C and D may cause more serious neuropathological symptoms than genotype A, it is increasingly important to follow which genotypes circulate in the community, and also to measure specific immunity against individual genotypes.

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