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Temporal and geographical distribution of measles virus genotypes

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The nucleotide sequence encoding the C terminus of the nucleocapsid protein of measles virus (MV) is the most variable in the genome. The sequence of this region is reported for 21 new MV strains and for virus RNA obtained from cases of subacute panencephalitis (SSPE) tissue. The nucleotide sequence of a total of 65 MV strains has been analysed using the CLUSTAL program to determine the relationships between the strains. An unrooted tree shows that eight different genotypes can be discerned amongst the sequences analysed so far. The data show that the C-terminal coding sequence of the nucleocapsid gene, although highly variable between strains, is stable in a given strain and does not appear to diverge in tissue culture. It therefore provides a good 'signature' sequence for specific genotypes. The sequence of this region can be used to discriminate new imported viruses from old 'endemic' strains of MV in a geographical area. The different genotypes are not geographically restricted although some appear to be the mainly 'endemic' types in large areas of the world. In global terms there appears to be at least four co-circulating genotypes of MV. The low level of divergence in the Edmonston lineage group isolated before 1970 indicates that some isolates are probably laboratory contaminants. This applies to some SSPE isolates such as the Hallé, Mantooth and Horta-Barbosa strains as well as some wild-type isolates from that period.

Introduction

Measles virus (MV) is an important human pathogen that still causes the death of over one million children per annum, primarily in the developing world. The serologically monotypic virus appears to have no animal reservoir and thus vaccination with the single serotype should theoretically be able to lead to eventual eradication. However, variation exists between virus strains in monoclonal antibody reactivity (Sheshberadaran *et al.*, 1983), plaque morphology and fusiogenicity (Rapp, 1964; Carrigan, 1986), temperature sensitivity (Bergholz *et al.*, 1975; Haspel *et al.*, 1975; Vydelinghum *et al.*, 1989) and ability to induce interferon (McKimm & Rapp, 1977), as well as in nucleotide sequence. These may be confounding factors in an eradication programme and thus the biological significance of this variation needs to be established.

The nucleotide sequence of two MV Edmonston-derived vaccine strains has been determined to be 15894 nucleotides in length (Crowley *et al.*, 1988, and corrections at accession number K01711 in the databanks; Komase *et al.*, 1995). The negative-stranded non-segmented RNA genome consists of six transcription units which generate the mRNAs for six structural proteins of the virus as well as at least two non-structural proteins (Barrett *et al.*, 1991). The MV structural proteins are the nucleocapsid protein (N), the phosphoprotein (P), the large protein (L), the matrix protein (M) and the two glycoproteins with the haemagglutination (H protein) and fusion activity (F protein). The mRNA for the P protein also encodes, in an overlapping reading frame, the non-structural C protein (Bellini *et al.*, 1985; mRNAs for the V protein are generated from the same locus by co-transcriptional insertion of two G residues at a site in the P gene which has similarity to that of the polyadenylation signal in the morbilliviruses (Cattaneo *et al.*, 1989*a*). Strain-specific changes in nucleotide sequence other than those associated with prolonged brain infections by the virus (Cattaneo *et al.*, 1989*b*) in the N, P and H genes do not affect the functionality of the proteins, in so far as these can be assessed, but reflect strain variation (Cattaneo *et al.*, 1989*b*). The original descriptions of the comparison of canine distemper virus (CDV) with MV had already indicated that certain areas of the genome of morbilliviruses have greater plasticity than others (Rozenblatt *et al.*, 1985; Bellini *et al.*, 1985).

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Table 1. *Origins of measles virus strains*

Group	Name	Description	Sequence reference	Accession number
A	Edm V	Edmonston-Enders vaccine/USA/1954	Rozenblatt *et al.* (1985)*	M10297
	Edm P9	SDA variant of Edm. strain⁻	Taylor *et al.* (1991)	D01001
	Edm B	Edm. B vaccine	Rota *et al.* (1994*a*)	U03656
	Edm wt	Edm. Wild-type/USA/1954	Rota *et al.* (1994*a*)	U01987
	Edm wtpf	Edm. Wild-type/USA/1954 plaque purified	This work	X84881
	Edm Zag	Edmonston-Zagreb vaccine	Rota *et al.* (1994*a*)	U03658
	Schw	Schwarz vaccine	Rota *et al.* (1994*a*)	U03668
	Hu2	Schwarz-vaccine-related case/N.Ireland/1971	Taylor *et al.* (1991)	D01011
	Mor 1	Moraten vaccine	Rota *et al.* (1994*a*)	U01999
	Mor 2	Moraten-vaccine-related case/Netherlands/1990	Taylor *et al.* (1991)	D01006
	CAM	Vaccine from Tanabe strain/Japan/1968	Rota *et al.* (1994*a*)	U03650
	Len	Leningrad-16 strain/Russia/1960	Rota *et al.* (1994*a*)	U03661
	S191	Shanghai vaccine/China/1960	Rota *et al.* (1994*a*)	U03664
	Chg	Changchun-47 vaccine/China/1957	Rota *et al.* (1994*a*)	U03653
	AIK-C	Edm.-derived vaccine	Mori *et al.* (1993)	K01711
	Hln	Halonen strain/Finland/1962	Rota *et al.* (1994*a*)	U01996
	Ph 26	Philadelphia-26 strain/USA/1957	Rota *et al.* (1994*a*)	U01991
	Hal	Hallé SSPE isolate/USA/1971	Buckland *et al.* (1988)	X13480
	Man	Mantooth SSPE strain/USA/1971	This work	X84875
	HB	Horta-Barbosa SSPE strain/USA/1971	This work	X84876
B1	Y22	Wild-type/Cameroon/1983	Taylor *et al.* (1991)	D01010
	Y14	Wild-type/Cameroon/1983	Rota *et al.* (1994*b*)	U01998
B2	R118	Wild-type/Gabon/1984	Taylor *et al.* (1991)	D01009
	R103	Wild-type/Gabon/1984	Rota *et al.* (1994*b*)	U01992
	R113	Wild-type/Gabon/1984	Rota *et al.* (1994*b*)	U01993
	R96	Wild-type/Gabon/1984	Rota *et al.* (1994*b*)	U01994
C1	MF	SSPE case/Europe/early 70s	This work	X84882
	SIP3A	SSPE case 1P3/USA/early 70s	Cattaneo *et al.* (1989*b*)	X16566
	S(A)	SSPE case A/Germany/mid 80s	Cattaneo *et al.* (1989*b*)	X16567
	YA	SSPE case Yamagata-1/Japan/late 80s	Yoshikawa *et al.* (1990)	NA
	SMa81	SSPE case/Madrid/1981/1970	This work	X84866
	S(K)	SSPE case K/Germany/mid 80s	This work	X84883
	Mad78	Wild-type/Madrid/1978	This work	X84867
	Mad79	Wild-type/Madrid/1979	This work	X84868
C2	JM	Wild-type/Bethesda USA/1977	Taylor *et al.* (1991)	D01002
	S(B)	SSPE case B/Austria/mid 80s	Cattaneo *et al.* (1989*b*)	X16568
	WTF	Wild-type/Germany/1990	This work	X84872
	Bil	Wild-type/The Netherlands/1991	This work	X84878
	DL	Wild-type/Germany/1992	This work	X84873
	LB	Wild-type/Germany/1993	This work	X84880
	Ma92A	Wild-type/Madrid/1992	This work	X84869
	Ma92R	Wild-type/Madrid/1992	This work	X84870
	Ma93F	Wild-type/Madrid/1993	This work	X84871
C3	SBI	Wild-type/Bonn Germany/1992	This work	X84874
D1	MVO	Wild-type/Bristol UK/1974	Taylor *et al.* (1991)	D01004
	MVP	Wild-type/Bristol UK/1974	Taylor *et al.* (1991)	D01005
	S33	SSPE/N.Ireland/1983	Taylor *et al.* (1991)	D01008
	S81	SSPE/N.Ireland/1986	Taylor *et al.* (1991)	D01007
D2	CL	Wild-type/Birmingham UK/1988	Schulz *et al.* (1992)	NA
	SE	Wild-type/Birmingham UK/1988	Schulz *et al.* (1992)	NA
	TT	Wild-type Kawasaki case/London/1991	Schulz *et al.* (1992)	NA
	Can	Wild-type/Canada/1989	Rota *et al.* (1994*b*)	U01976
	Chil	Wild-type/Chicago USA/1989	Rota *et al.* (1994*b*)	U01977
	Chi2	Wild-type/Chicago USA/1989	Rota *et al.* (1994*b*)	U01978
	SD	Wild-type/San Diego USA/1989	Rota *et al.* (1994*b*)	U01995
	JK	MIBE/USA/1990	Rota *et al.* (1994*b*)	U01988
D3	Ma94B	Wild-type/Madrid/1994	This work	X84863
E	Brx	Encephalitis case/Germany/1971	This work	X84879
	WFK	Wild-type/Woodfolk USA/early 70s	This work	X84877
	CM	Wild-type/USA/late 70s	Taylor *et al.* (1991)	D01003
	S(C)	MIBE/USA/late 70s	Cattaneo *et al.* (1989*b*)	X16569
F	SMa79	SSPE case/Madrid/1979/1967	This work	X84864
	SMa94	SSPE case/Madrid/1994/1968	This work	X84865
G	Be83	Wild-type/Berkeley USA/1983	Rota *et al.* (1994*b*)	U01974
	B083	Wild-type/Boston USA/1983	Rota *et al.* (1994*b*)	U01990

* Corrected as described in Rota *et al.* (1994*a*).
NA, Not available.

From comparisons and alignments of the major structural proteins of paramyxo- and morbilliviruses it has become clear (Rima, 1989) that the C-terminal part of the nucleocapsid gene and the N-terminal 100 amino acids of the P/C protein (Baczko *et al.*, 1992) are the most variable parts of the genomes in this group of viruses. Although all six genes of MV appear to vary, though to different extents (Baczko *et al.*, 1991, 1992; Rota *et al.*, 1992; Komase *et al.*, 1995), the highest degree of variation is in the C terminus of the N protein and in the H and P proteins. The sequence of the C-terminal 151 amino acids of the N protein has been analysed in 18 strains of MV (Taylor *et al.*, 1991): there was up to 7·2% divergence in the nucleotide sequence and 10·6% divergence in the amino acid sequence between the most unrelated strains in this region and a number of specific lineage groups of MV were shown to exist. In this report we have used this region to further analyse and group the genotypes of MV. We show that some genotypes have a long history of circulation in the human population, that others can no longer be isolated from wild infections and that several genotypes are not geographically restricted as suggested previously, but can be isolated across the world. Two new genotypes have been found.

Methods

Virus strains. Table 1 shows the origin of the strains analysed in this paper in addition to the ones described and analysed earlier. In order to obtain virus RNA, Vero cells or cell lines of lymphoblastoid origin (B95-8) were infected, or in the case of persistent infection *in vivo* (i.e. brain) or *in vitro* (i.e. tissue culture) sequences were determined directly by reverse transcription followed by polymerase chain reaction (RT–PCR) on RNA samples extracted from infected tissue or cells using guanidinium isothiocyanate as described by Taylor *et al.* (1991).

RT–PCR. RNA was extracted from infected tissues or cultured cells (see above) using the guanidinium isothiocyanate technique. The RNA was then reverse transcribed using mouse Moloney virus reverse transcriptase and oligo(dT) as primer. The reaction was initiated at 37 °C. After reverse transcription specific primers (1197, 5′ ATTAGGGCAAGAGATGGTAAGG 3′; 1723, 3′ GGGAGGTAGTAACAATAT 5′) were added and PCR was carried out as described by Taylor *et al.* (1991).

Cloning and nucleotide sequence determination. The PCR products were subcloned into the pGem-T vector system (Promega) or sequenced directly. The nucleotide sequence was determined using dideoxy chain-terminators with M13 forward and reverse primers as described by Sanger *et al.* (1975). Sequence data were analysed with the CLUSTAL program (Higgins & Sharp, 1988) using 1000 bootstrap replicates of neighbour-joining trees.

Results

In the present study we report the nucleotide sequences of 21 strains of MV in addition to the ones used to construct previous dendrograms (Taylor *et al.*, 1991) and those analysed by Rota *et al.* (1994*a*, *b*). We also included the sequences of other wild-type strains reported by Schulz *et al.* (1992) and Yoshikawa *et al.* (1990). This extended the number of sequences analysed for the coding region of the hypervariable part of the N protein to 65. The origin and derivation of the viruses together with sequence references and accession numbers are given in Table 1. The nucleotide sequence of the Edmonston-Enders strain of MV (Rozenblatt *et al.*, 1985), which had been included in the previous paper, was amended, taking account of sequencing results obtained when the Edmonston-derived vaccines were compared with a plaque-purified wild-type strain Edmonston (Edm wtpf) isolate obtained from Professor A. D. M. E. Osterhaus (Rotterdam, The Netherlands). These corrections were identical to those suggested by Rota *et al.* (1994*a*), so that consensus now exists about the correct sequence of the Edmonston-Enders vaccine strain. A number of other MV isolates were also included. Some older isolates that had not been sequenced before included the Woodfolk isolate, a wild-type virus from the USA isolated in the early 1970s and the Braxator virus isolated from a measles encephalitis case in Germany in 1971. Also included was the sequence of the subacute sclerosing panencephalitis (SSPE) case K reported previously (Baczko *et al.*, 1984). The Mantooth and Horta-Barbosa isolates (Thormar *et al.*, 1978) appear to be the same virus isolated from the brain of an SSPE patient in 1971. These two viruses have reached our laboratories via different intermediaries, have a widely different passage history, but are essentially the same in nucleotide sequence barring one change at position 1459. The other isolates for which sequence information is newly reported here are the Bilthoven (Bil) virus obtained in an outbreak in The Netherlands by Professor A. D. M. E. Osterhaus and colleagues (van Binnendijk *et al.*, 1994) and several German isolates, including: wild-type Fleckenstein (WTF) made in 1990 in Erlangen; DL made in 1992; LB made in 1993 and SBI made in Bonn in 1992. Sequence data from a large number of Spanish wild-type isolates made in Madrid from MV cases in 1978 (Mad78) and 1979 (Mad79), case AOM in 1992 (Ma92A), case RMS (Ma92R), case FV in 1993 (Ma93F) and case BCL in 1994 (Ma94B) were also included. Furthermore, the sequences of three Spanish SSPE cases were also analysed. Case 791520 (SMa79) was autopsied in 1979 after a well-recorded history of MV infection in 1967. Case EV (SMa94) was a 27-year-old woman who died in 1994 after infection with measles in 1968, having had symptoms since 1977. Case 812780 (SMa81) died in 1981 after a history of measles in 1970.

The nucleotide sequences determined for this paper and not published previously are given in Fig. 1, together with a consensus sequence of the 456 nucleotides used in this analysis. For the other strains the sequences of 456 nucleotides between residue numbers 1230 and 1685 of

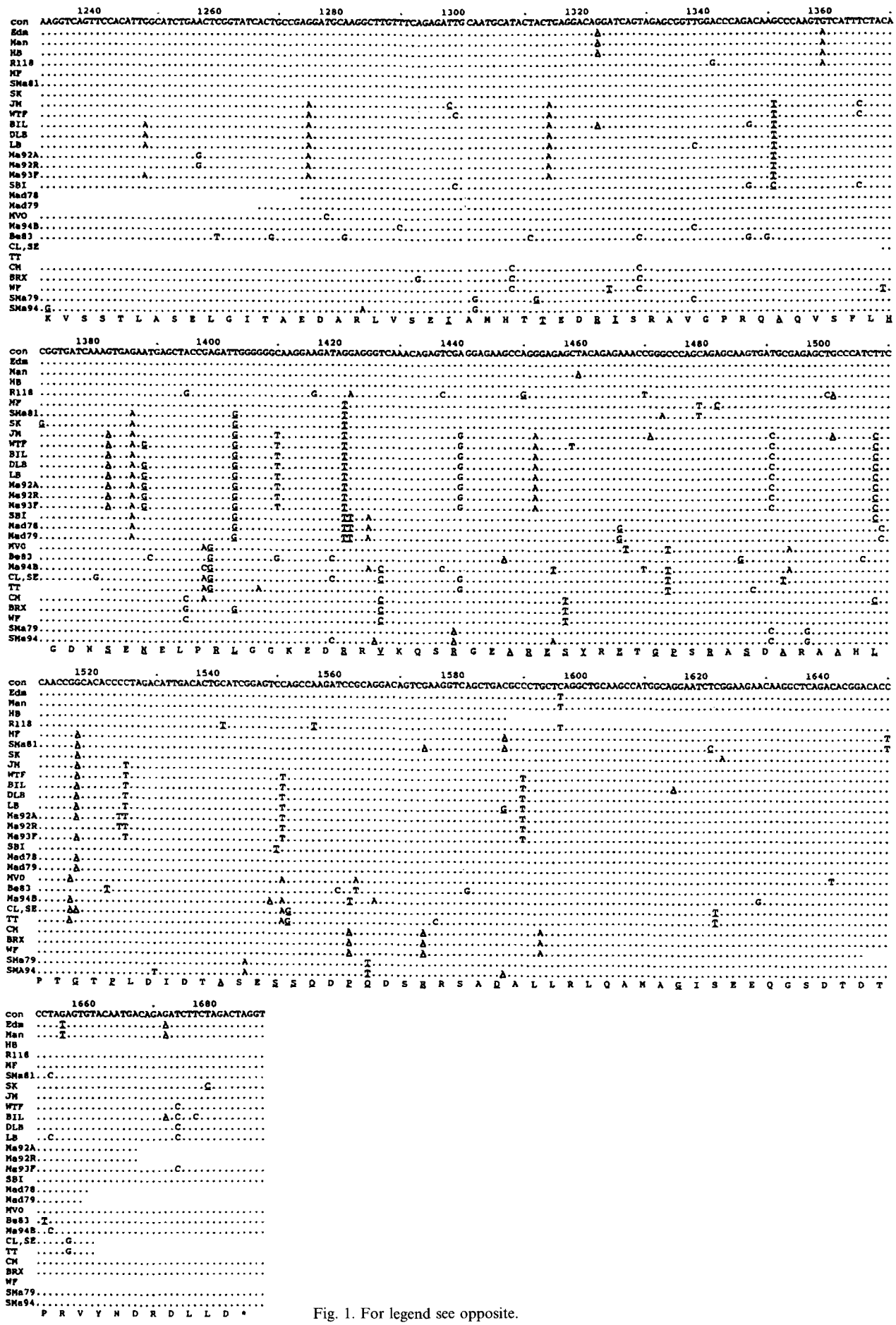

Fig. 1. For legend see opposite.

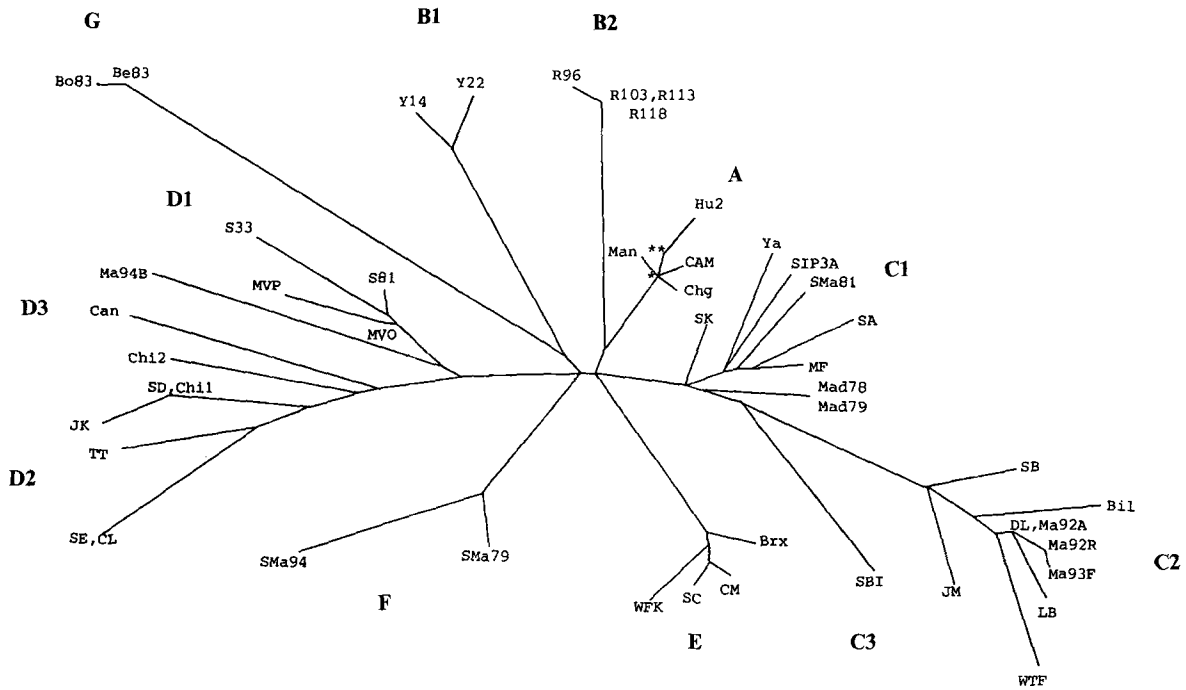

Fig. 2. Unrooted tree diagram of the relationships between 65 MV strains. The tree was drawn on the basis of the CLUSTAL program. Strain abbreviations and groupings are as described in Table 1. Identical sequences are indicated by asterisks (*, Edm wt, wtpf, vac, AIK-C, Zag, Ph 26, Hln, Len, HB, Hal; **, Edm B, Schw, Morl, Mor 2, Edm P9, S191).

the genome, numbered according to the AIK-C sequence of Mori *et al.* (1993), were taken from the GenBank and EMBL databases, when available, and aligned without gaps. An unrooted tree shown in Fig. 2 was constructed as described above. The same diagram with eight branches was generated in four independent analyses carried out at different stages of construction of the tree. However, the four central nodes of the tree have less significance as their arrangement differed between analyses. This occurs because the central nodes of the trees are not determined by any real virus sequences but are artificial constructs of the program. This tree analysis showed clearly that the virus isolates fell into a number of groups. This grouping was used in the construction of Table 1. The names of the groups conform to those of our earlier paper (Taylor *et al.*, 1991).

Discussion

It is clear that the hypervariable region coding for the C-terminal 151 amino acid residues of the nucleocapsid protein of MV fulfills the requirements of a good signature sequence for strain differentiation. First, it is the most variable part of the genome and although theoretically its position near the 3′ end of the genome and potential recombination might reduce its usefulness as an indicator of variation in the whole genome, the lack of evidence for recombination in MV or other members of the Order *Mononegavirales* removes this objection. Secondly, the sequence appears to be very stable as no changes occurred during adaptation of MV strains to growth in cells of either monkey (Vero or B95-8) or human origin (BJAB). This stability is also demonstrated by the sequences of the Hallé, Mantooth and Horta-Barbosa strains. The latter two appeared from the available literature to be the same strain and were found to have only one nucleotide change despite a widely divergent passage history. In the case of the Horta-Barbosa strain, in our laboratory several plaque purifications and separation into large and small plaque types (Gould *et al.*, 1976) did not appear to have changed the sequence significantly.

The criteria by which it would be decided that a

Fig. 1. Alignment of nucleotide sequences of various MV strains. The consensus sequence (con) is determined from all 65 sequences now used in the analysis. The sequences of the strains newly described in this work are given. Underlined nucleotide changes give rise to amino acid replacements of the underlined residues in the protein consensus (given in single-letter code). A dot indicates the same residue as in the consensus. Some sequences have not been determined over the entire region. Abbreviations for strains are as described in Table 1.

particular strain represents a new genotype or not have not been determined and thus, somewhat arbitrarily, the eight branches emanating from the four central nodes in Fig. 2 have been chosen to represent distinct genotypes as they are more than three nucleotide changes away from the central nodes. Comparison with the earlier reported groupings shows that only two new groupings appeared. One is represented by two Spanish SSPE case sequences (group F) and the other (group G) by two American wild-type isolates from 1983 (Boston 83 and Berkeley 83). Also, the B group which originally consisted of two widely diverging African isolates has, in the new trees, been split into two independent branches. The number of different MV genotypes is eight at present but, in order to establish the real distribution and number, more sampling from Africa and the Near-East and Far-East Asia will have to be carried out.

Group A comprises all the vaccine viruses identified so far. The majority of these have been derived from passage of the Edmonston B virus (Rota *et al.*, 1994*a*) but other vaccine strains such as the CAM, Leningrad 16, Shanghai and Changchun vaccines are all related to the Edmonston strain (Rota *et al.*, 1994*a*). This group also contains a number of wild-type isolates made before 1970 (e.g. the Halonen and Philadelphia isolates; Rota *et al.*, 1994*a*) but also some suggested SSPE isolates such as the Mantooth, Hallé and Horta-Barbosa strains. It is possible that this group was very widely distributed throughout the world in the early 1950s and that these isolates represent true wild-type strains from SSPE cases. However, the fact that all the SSPE-derived viruses which are defective in growth together with virus sequences determined directly from brain RNA fall into other genotype groups may indicate that those tissue-culture-adapted, good-growing viruses represent laboratory contaminants rather than true SSPE isolates. This question is important in relation to the possible association of vaccination with the subsequent development of SSPE. Another interesting fact commented on previously (Rota *et al.*, 1994*a*) is that all the vaccines used at present appear to fall into this group. This, coupled with the fact that the Halonen and Philadelphia strains, which were isolated before 1970, are also in this group, prompted Rota *et al.* 1994*a*) to suggest that vaccination may have driven the variation observed between MV strains and that the Edmonston genotype may have been a world-wide genotype before vaccination started. However, this would be surprising when one considers the length of time which, for example, the C and D genotypes appear to have been in circulation (more than two decades). Furthermore, the fact that mutation rates do not appear to be very large is difficult to reconcile with the suggestion that before 1967 all MV was of the Edmonston genotype.

The level of variation in this genotype over the years between first isolation in 1954, subsequent isolations made in 1957, 1960, 1962 and 1968, and the last recorded isolation in 1971 is nil or one nucleotide in the sequenced region and negligible in comparison to that observed in the other groups (Fig. 2). Hence, we prefer to suggest laboratory contamination as an alternative and more plausible explanation for the observed close relationship between the isolates in group A.

There appeared to be no differences between the Edm. wild-type, the plaque-purified version of the Edmonston wild-type virus obtained from Professor A. D. M. E. Osterhaus (Rotterdam, The Netherlands), and vaccine strains. Furthermore, the Moraten-vaccine-associated case (Mor 2) and the Schwarz-vaccine-associated case (Hu2) revealed no common mutations (Taylor *et al.*, 1991). Thus in this region of the genome there appear to be no mutations of direct significance for the attenuated phenotype and the differences between the more attenuated viruses (Moraten and Schwarz) and the earlier Edmonston vaccines are probably coincidental with changes elsewhere. These results are in agreement with those of Rota *et al.* (1994*a*).

Group B comprises African isolates from two independent epidemics in Cameroon and Gabon in 1983 and 1984, respectively. The isolates made within each country do not differ significantly as the strains sequenced by Rota *et al.* (1994*b*) appeared to be identical or have only a small number of nucleotide changes with respect to the R118 and Y22 sequences determined previously (Taylor *et al.*, 1991). The distance from the central nodes to the actual sequence of the isolates from the two countries is large and the latest tree (Fig. 2) shows these as two independent branches referred to as group B1 (Cameroon isolates) and B2 (Gabon isolates). The effect of vaccination programmes on the circulation of such strains and their continued presence in Africa should be examined further.

The third genotype (group C) is split (on the basis of the unrooted tree) into three distinct subgroups: C1, C2 and C3. The C1 group is typified by the MF strain and comprises two defective strains propagated as persistent infections in tissue culture (MF and SIP3A), three cDNA sequences derived directly from SSPE brain RNA from cases in which virus has not been isolated [S(A), S(K) and SMa81], one defectively growing strain (Yamagata-1) and two wild-type isolates from Spain in 1978 and 1979. One further case of SSPE also belongs to this group on the basis of the sequences of the M gene, i.e. the Biken isolate of SSPE made in 1975 in Japan (Wong & Hirano, 1987). The grouping of various strains appears to be independent of the genes used for the analysis (Baczko *et al.*, 1991, 1992; Rota *et al.*, 1992, 1994*a*, *b*; Komase *et al.*, 1995). The last representative of this

group was identified in the late 1980s as an SSPE-derived isolate from Japan, presumably reflecting a much earlier MV infection, and no further isolations have been made of related viruses. The fact that this group contains mostly SSPE isolates probably reflects the poor isolation history of MV rather than a specific property of these sequence variants. The C2 group typified by strain JM has been isolated from the USA only once in the late 1970s and since then has been reported in the 1980s from Germany and Austria and since 1990 every year in Germany (strains WTF, DL and LB), in Holland in 1991 (Bilthoven virus) and in 1992/93 in Spain (Ma92A, Ma92R and Ma93F). Whether this group originated in the USA or whether the 1977 isolation reflected the import of a European genotype is not clear and again the lack of comparator strains does not allow this question to be answered. This group circulates currently in populations in which a high vaccine coverage has been achieved and is now responsible for isolated outbreaks in several European countries. Presumably, the presence of sufficient numbers of new-born infants, primary and secondary vaccination failures and groups that refuse vaccination provides a reservoir of susceptible hosts for the continued circulation of these viruses. In 1992 a new virus, strain SBI, was isolated near Bonn in Germany. It appeared to constitute an offshoot of the C group via a node that splits the main branch between the C1 and C2 groups. Its origin is unclear. Most likely it represents an imported virus genotype rather than a drastically mutated form of the C group viruses. Its occurrence would indicate that similar viruses must have been circulating elsewhere in the world in 1992.

The D group has now been split into several subgroups. The D1 group comprises the four isolates related to the MVO strains of 1974 from Bristol in the UK. The D2 subgroup appears to be related to these viruses, but since no isolates are available on the nodes of the branch points of the unrooted trees, it is difficult to establish the correct relationships between groups of genotypes. The first isolations of the D2 group were made in the UK in 1988 and this genotype was still circulating in the UK in 1991 whereas related strains appeared in Canada in 1989 and caused the well-researched outbreak in the USA in 1989/90 (Rota *et al.*, 1994*b*). The relationships depicted here for the N genes are similar to those found in the M and H genes (Rota *et al.*, 1994*b*).

Group E, with the Woodfolk strain as earliest representative, has been isolated three times in the USA and once in Germany in 1971. It does not appear to be circulating at present in either country and may be extinct. The relationships for the P and M and H genes supported the distinct nature of this group (Baczko *et al.*, 1991, 1992, and unpublished).

Group F consists of two sequences obtained from MV RNA from the brains of two SSPE patients (SMa79 and SMa94) who had documented histories of primary measles infection in 1967 and 1968. These cases provide support for the hitherto unproven hypothesis that the wild-type virus circulating at the time of onset of SSPE is not the one that gives rise to the persistent infection, as the MV strains circulating in Spain at the onset of symptoms of one case and the death of the other belonged to group C, which circulated from 1970 (SMa81) to 1978/79 (Mad78 and Mad79; group C1) and to 1992/93 (Ma92 and Ma93; group C2), whereas the virus found in the brains of the two patients belonged to type F. Thus, even though the patients died with an interval of 15 years, the persistent virus belonged to a type which probably circulated in 1967/68 when both patients had their original primary infection. In 1994 a virus from the D group was isolated in Spain, signifying either an imported strain or a further shift in the genotype circulating in that country.

Group G comprises only two single isolates from the USA in 1983 sequenced by Rota *et al.* (1994*b*). These appear to have no relationship to any of the other groups and again it is not clear whether they represent imports from a group circulating elsewhere or an extinct lineage group. They provide the largest branch distance in the tree (Fig. 2).

In summary, the analysis presented here demonstrates the existence of at least eight genotypes of MV, depending on the criteria that are used to separate the various groups. Virus genotypes A, E and F have not been isolated recently from wild-type measles infections. They indicate that there are two main genotypes, in groups C2 and D2, that are circulating at present but that the isolation records need to be improved to determine whether other wild-type genotypes have evolved from the African groups (B1 and B2). The origin of the Ma94B (group C) and SBI (group D) strains is enigmatic, as they represent viruses that derive from nodes that link 'early'. Their recent isolation indicates that other genotypes are circulating and that we do not have a complete overview of the current MV strains. It will be important in any eradication campaign to monitor the distribution of viruses and hence it is clear from these data that surveillance of MV strains needs to be developed further.

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