Molecular differences between two Jeryl Lynn mumps virus vaccine component strains, JL5 and JL2

Phil Chambers, Bert K. Rima and W. Paul Duprex

Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Medical Biology Centre, Queen’s University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

Correspondence
Bert K. Rima
b.rima@qub.ac.uk

The Jeryl Lynn (JL) vaccine against mumps virus (MuV) contains two components, MuV\textsubscript{JL5} and MuV\textsubscript{JL2}, which differ by over 400 nt. Due to the occurrence of bias in the direction of mutation, these differences and those found in nucleotide sequences of different isolates of the minor component in the vaccine (MuV\textsubscript{JL2}) might be due to the effect of ADAR-like deaminases on MuV grown in tissue-cultured cells. A molecular clone of MuV\textsubscript{JL2} (pMuV\textsubscript{JL2}) and MuV\textsubscript{JL2}-specific helper plasmids were constructed in order to investigate molecular interactions between MuV\textsubscript{JL5} and MuV\textsubscript{JL2}, to augment the existing molecular clone of MuV\textsubscript{JL5} (pMuV\textsubscript{JL5}) and MuV\textsubscript{JL5}-specific helper plasmids. Genome and mRNA termini of MuV\textsubscript{JL2} were characterized, and an unusual oligo-G insertion transcriptional editing event was detected near the F mRNA polyadenylation site of MuV\textsubscript{JL2}, but not of MuV\textsubscript{JL5}. Genes encoding glycoproteins of rMuV\textsubscript{JL2} and rMuV\textsubscript{JL5} have been exchanged to characterize the oligo-G insertion, which associated with the specific sequence of the F gene of MuV\textsubscript{JL2} and not with any other genes or the RNA-dependent RNA polymerase of strain MuV\textsubscript{JL2}. The results indicate that a single G-to-A sequence change obliterates the co-transcriptional editing of the F mRNA and that this oligo-G insertion does not affect the growth of the virus.

INTRODUCTION

The Jeryl Lynn (JL) mumps virus (MuV) vaccine contains two different component strains, MuV\textsubscript{JL5} and MuV\textsubscript{JL2}, that differ considerably in their nucleotide sequences (Amexis \textit{et al.}, 2002). The mechanisms that generated the two variants are unclear. MuV belongs to the genus \textit{Rubulavirus} of the subfamily \textit{Paramyxovirinae}. The inner core consists of the non-segmented negative-strand viral RNA (length, 15 384 nt) associated with the nucleocapsid (N) protein, lesser amounts of the polymerase associated phospho- (P) protein and small amounts of the large (L) RNA-dependent RNA polymerase (RdRp) protein. In the virion, the ribonucleoprotein (RNP) is surrounded by a host-derived membrane, which is spanned by two glycoproteins: the haemagglutinin–neuraminidase (HN) and fusion (F) proteins. On the inner face of the membrane is a membrane or matrix (M) protein that interacts with the cytoplasmic tails of the HN and F proteins and with the RNP. The virus also expresses three non-structural proteins. Two are derived from the second transcription unit, which encodes the non-structural V protein directly and, after co-transcriptional editing of the transcripts, gives rise to two other proteins, P and W. The W protein is a second non-structural protein consisting of a truncated version of V, which substitutes a short peptide of unknown function, encoded by the third potential reading frame, for the cysteine-rich tail that is characteristic of the V proteins of all members of the \textit{Paramyxovirinae}. The third non-structural protein is a small hydrophobic (SH) protein that is derived from a transcription unit between those encoding the F and HN proteins (Elliott \textit{et al.}, 1989; Elango \textit{et al.}, 1989). Although some aspects of the replication of MuV have been studied at the molecular level, others have been inferred only by analogy to other members of the \textit{Paramyxovirinae}. For example, the genome and 5' transcriptional termini of MuV have been generally inferred from sequence comparisons rather than determined directly. The mRNA 3' termini have been better characterized by sequencing cDNA clones generated by oligo-dT priming on MuV mRNA (Elango \textit{et al.}, 1988). Furthermore, polyadenylation and polymerase slippage at the 3' end of the genes and at the editing site have not been subjected to rigorous analysis, and direct proof of the inferred sequence determinants is lacking. The development of reverse-genetics systems (Amexis \textit{et al.}, 2002; Lemon \textit{et al.}, 2007) now allows many of the basic parameters of MuV replication to be studied in detail.
The MuV JL vaccine has been reported to be derived from a single clinical isolate (Afzal et al., 1993), which was converted into a live-attenuated vaccine by passage of virus in non-human host cells. The complete nucleotide sequences of MuVIL5 and MuVIL2 have been reported (Clarke et al., 2000; Amexis et al., 2002) and show 414 nucleotide changes, resulting in 87 amino acid changes, between MuVIL5 and MuVIL2. This level of variation is at the same level as differences between genotypes of MuV. There are biological differences between MuVIL5 and MuVIL2. For example, MuVIL2 grows better in embryonated eggs than MuVIL5, but immunological differences have not been reported (Amexis et al., 2002). Both MuVIL5 and MuVIL2 are non-neurovirulent in the rat neurovirus-test (Rubin et al., 1999, 2000, 2003). Molecular differences other than the nucleotide sequences have not been investigated extensively.

METHODS

Virus strains and cell-culture procedures. A549 and Vero cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% (v/v) fetal calf serum (FCS; Invitrogen). MuV strains MuVIL5, MuVIL2 and MuVIL1 were obtained from Dr D. Clarke (Wyeth-Lederle Vaccines, Pearl River, NY 10965, USA), Dr M. Afzal (National Institute for Biological Standards and Control, Potters Bar, London, UK) and Dr S. A. Rubin (Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA), respectively. Modified vaccinia virus Ankara (MVA-T7) was grown as described previously (Duprex et al., 1999). For virus growth curves, Vero cells were grown in 75 cm² bottles and infected at 50% confluence at an m.o.i. of 0.01, and maintained in fresh DMEM/10% FCS. TCID50 titres were determined in triplicate (1200

Bacterial strains and plasmids. Escherichia coli strain DH5aIQ was used for routine transformations; highly competent E. coli strains XL blue and TOP10 were obtained from Stratagene and Invitrogen, respectively, and used where fewer transformants were expected. The full-length MuV clone pMuVFL encoding MuVIL5, helper plasmids encoding MuVIL5 N, P and L proteins and pMuVDICAT were obtained from Dr D. Clarke (Wyeth-Lederle Vaccines, Pearl River, NY 10965, USA). The cytomegalovirus (CMV) promoter-based expression plasmid pCG (Cathomen et al., 1995) was used as the basis for construction of MuVIL2-specific helper plasmids pCG-NIL2, pCG-PIL2 and pCG-LIL2.

Reagents. Oligonucleotides were obtained from Qiagen. TRIzol reagent was from Invitrogen. Restriction enzymes, reverse transcriptase SuperScript III, high-fidelity Taq DNA polymerase, Pfu polymerase, Phusion DNA polymerase, Klenow fragment of DNA polymerase, exonuclease III and DNA ligase were obtained from New England Biolabs (NEB) or Invitrogen and used according to the manufacturers’ instructions.

Recombinant DNA manipulations. PCR products corresponding to MuV genes were generated with unique terminal restriction-enzyme sites and cloned into suitable intermediate vectors to generate two vectors containing approximately half of the MuVIL2 genome. One half comprised the leader to the N gene and was constructed in pUC18 by sequential ligations of genes from MuVIL2. The other half, comprising the L gene, was amplified in two overlapping segments and cloned sequentially into a modified form of the pBluescript-derived pMuVIL2 by deletion from N to HI, i.e. from an Sphi site near the start of the N gene to an Nhel site near the end of the N gene, thus replacing the L gene of MuVIL2 with that of MuVIL2. In the final stage, the leader to HI region of MuVIL2 from the pUC18-based intermediate was cloned into the pBluescript/MuVIL2 L gene intermediate to generate a full-length clone of strain MuVIL2. Details of oligonucleotides, enzymes etc. can be supplied on request.

The initial full-length clone of MuVIL2 was modified by rounds of in vitro mutagenesis of subgenomic clones, which were transferred to the full-length clone to insert further restriction sites at intergenic locations or to eliminate them elsewhere. Recombinant DNA operations were initially performed by standard RT-PCR, restrictions and ligations according to the manufacturers’ protocols, but a simple form of ligation-independent cloning (Aslanidis & de Jong, 1990; Li & Evans, 1997) was used for most procedures after the generation of the first full-length clone of MuVIL2. In brief, 1–200 ng restricted vector and 1–200 ng desalted PCR product (generated using oligonucleotides with termini homologous with vector and a DNA polymerase that generates blunt-ended PCR products) for insert were incubated for 10 min at 37 °C in a final volume of 10 μl of NEB buffer 1 (or the buffer with the lowest ionic strength consistent with digestion of vector) with 10 units exonuclease III. Next 2 μl 1 M NaCl was added and exonuclease III was heat-inactivated at 75 °C for 15 min. The reaction mixture was cooled slowly from approximately 55 to 37 °C in about 1.5 h in an insulated beaker of water and transformed into competent E. coli. The final full-length clone of MuVIL2 was designated pMuVIL2 and has restriction sites with blunt ends or 5’ restriction overhangs engineered in all intergenic positions, internal to all viral genes except SH and spaced evenly throughout L, because exonuclease III is reliable for 3’–5’ digestion of such restriction sites. The clone of the HI gene of MuVIL2 to the pMuVIL2 vector involved cloning to an Sphi site with a 3’ overhang on which exonuclease III is inactive. Klenow fragment of DNA polymerase, which has both 5’–3’ and 3’–5’ exonuclease activities, was used instead of exonuclease III for digestion of DNA during this procedure. In vitro mutagenesis was also performed by exonuclease III digestion of overlapping blunt-ended PCR termini generated with mutagenic oligonucleotides followed by annealing as above. Smaller plasmids (i.e. any gene except L in the initial small plasmid cloning vectors) could be mutated from a single PCR product, but longer DNAs (e.g. of the L gene half-genome clones) were generated as two overlapping pieces from the site of mutation to a site in the ampicillin-resistance gene of the vector.

Determination of MuV RNA termini by rapid amplification of cDNA ends (RACE). The 5’ termini of all MuV mRNAs and both genomic termini were determined by RACE after PCR using G-tailed cDNAs with a negative-sense gene-specific primer located close to the gene start for each mRNA (that for N generated two termini – that of the N mRNA and that of the antigenome; that for HI generated termini for both HI and SH) or a positive-sense primer located close to the end of the L gene for the 5’ terminus of the genome, and a common oligo-dC tailed primer as described previously (Barr et al., 1994). The 3’ termini of F gene mRNAs were determined in similar fashion after PCR using oligo-dT-primed cDNAs with a positive-sense primer located near the end of the F gene and an oligo-dT-tailed primer. Nucleotide sequences of all RNA termini were determined directly from the PCR products.

Rescue of recombinant viruses from cDNA clones. Initial rescue by transfection of full-length and helper plasmids to MVA-T7-
infected A549 cells, followed by overlay with Vero cells (Clarke et al., 2000), was used for rescue of all recombinant viruses. When MuV\textsuperscript{JI2} helper plasmids were used, 2 μg pCG-N\textsuperscript{JI2}, 1 μg pCG-P\textsuperscript{JI2} and 0.1 μg pCG-L\textsuperscript{JI2} substituted for the MuV\textsuperscript{JI5} analogues. Rescued recombinant viruses were verified by nucleotide sequence analysis of PCR-amplified viral genes using amplification without reverse transcription as a negative control.

RESULTS

Sequence variation between MuV\textsuperscript{JI2} and MuV\textsuperscript{JI5} is extensive

Numerous clone-to-clone variations were detected when the PCR products corresponding to MuV\textsuperscript{JI2} genes were sequenced and, in most instances, it was difficult to determine whether these were generated in the RT-PCR or whether they reflect genuine quasi-species variations in the virus population. The final consensus full-length sequence is available under GenBank accession no. FN431985. The numbers of differences between our consensus MuV\textsuperscript{JI2} sequence, the published consensus MuV\textsuperscript{JI2} sequence and the consensus sequence of strain MuV\textsuperscript{JI5} (Clarke et al., 2000; Lemon et al., 2007) are shown in Table 1. Three clusters of variations between our MuV\textsuperscript{JI2} sequences and those published were present and may be the result of hypermutation events, as they were all of the form where T (in the cDNA sequence) in one sequence was substituted by C in the other. One cluster is in the 3’ non-coding region of the L gene (nt 15254–15317) where there are six changes in 64 bases, of which five are T in the published MuV\textsuperscript{JI2}, but are C in our sequence – MuV\textsuperscript{JI5} has T at all these positions. The second region is in the P gene (nt 2217–2323) where there are seven changes in 107 bases, of which six are C in the published MuV\textsuperscript{JI2}, but are T in our sequence – MuV\textsuperscript{JI5} has T at these positions. The third region (nt 2459–2582) is also in the P gene (and was variable within our own series of clones) where there are three changes in 126 bases, all of which are T in the published MuV\textsuperscript{JI2} sequence and in one of our cDNA clones, but are C in two other cDNA clones – MuV\textsuperscript{JI5} has T at these positions. These three clusters contain 16 changes in 297 bases, a divergence of 5.4%; this is very high compared with the remainder of these full-length sequences, where there are 35 changes in 14987 nt, a divergence of 0.2%, and thus contributes substantially to the overall genomic sum of 51 changes in 15384 bases, a divergence of 0.3%. A further potential biased mutation event that may have occurred during passage of MuV\textsuperscript{JI2} and MuV\textsuperscript{JI5} is located in the 3’ non-coding region of the N gene (nt 1804–1870) where there are nine changes between MuV\textsuperscript{JI5} and both MuV\textsuperscript{JI2} sequences, of which eight are T in both of the MuV\textsuperscript{JI2} sequences, but C in MuV\textsuperscript{JI5}. A tenth change (nt 1820) in this region is present only in our MuV\textsuperscript{JI2} sequence, where T replaces C of the other two sequences.

Table 1. Nucleotide and amino acid differences between MuV\textsuperscript{JI5} and MuV\textsuperscript{JI2}

Values are shown as number of changes (percentage difference). MuV\textsuperscript{JI2} db is the sequence from Amexis et al. (2002); MuV\textsuperscript{JI5} cons is our consensus sequence.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Length (aa)</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MuV\textsuperscript{JI5}/MuV\textsuperscript{JI2}</td>
<td>MuV\textsuperscript{JI5}/MuV\textsuperscript{JI2} cons</td>
</tr>
<tr>
<td>Nucleotide</td>
<td>414 (2.7)</td>
<td>421 (2.7)</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>550</td>
<td>7 (1.3)</td>
</tr>
<tr>
<td>P</td>
<td>392</td>
<td>12 (3.0)</td>
</tr>
<tr>
<td>V</td>
<td>225</td>
<td>7 (3.1)</td>
</tr>
<tr>
<td>M</td>
<td>376</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>F</td>
<td>539</td>
<td>13 (2.4)</td>
</tr>
<tr>
<td>SH</td>
<td>58</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>HN</td>
<td>583</td>
<td>14 (2.4)</td>
</tr>
<tr>
<td>L</td>
<td>2262</td>
<td>13 (0.6)</td>
</tr>
</tbody>
</table>
In an early study (Elango et al., 1988), the mRNA 3’ termini and polyadenylation signals of all genes except L were identified directly in the SBL1 strain of MuV (MuVSBL) and located in tracts of six and seven adenosine residues (positive-sense sequence). We directed our attention to the 3’ end of the F gene of MuVJL2 where there are two such tracts separated by only seven intervening bases, one of six and one of seven adenosine residues, which are both rather similar to potential polyadenylation-site motifs. There is also sequence divergence between strains MuV JL2 and MuVJL5 with three changes out of eight bases in part of the region comprising the two polyadenylation motifs (Fig. 2). In addition, the Enders strain of MuV (MuVEnders) expresses only F–SH read-through transcripts and has an A-to-G mutation in the region comprising the seven-adenosine tract of the other MuV strains (Takeuchi et al., 1991). We mapped the 3’ end of the F mRNA by RACE to determine which polyadenylation motif (or neither, or both) might be used during mRNA synthesis for MuVJL2. All polyadenylation of the F gene of MuVJL2 occurred at the second motif (Fig. 3) in the seven-adenosine tract at the end of the F gene, which is also used for polyadenylation of the MuVJL5 F mRNA.

### Fig. 1. Location of MuVJL2 genome termini and mRNA 5’ termini.

Sequence chromatograms that identify MuVJL2 genome termini and mRNA 5’ termini. In (a–h), the orientation of the chromatograms has been reversed so that all sequences can be read in the standard positive direction. Ten bases of tail/cap artefact and 10 bases of viral sequence are shown for each. (a, i) Genome 5’ and 3’ termini; (b–h) 5’ termini of the N, P/V, M, F, SH, HN and L mRNAs, respectively. Pairs of sequences (a) and (b) or (f) and (g) were taken from the same electrochromatograms. Arrowheads in (b–h) indicate the base inserted complementary to the mRNA methylated guanosine cap structure – this is predominantly C in the cDNA copy and thus appears as G in this orientation. The peak signals in the M mRNA start (d) were higher than optimal, generating artefact peaks under the tail. The F mRNA start (e) also contains a minor signal from the M–F gene junction. The HN mRNA start (g) contains a signal from an abundant SH–HN transcript of approximately equal intensity to the tail itself (Afzal et al., 1990).

### Fig. 2. Potential polyadenylation motifs at the 3’ UTR of the F gene of MuVJL2

Although the first of the two potential polyadenylation signals did not appear to be used, it did allow slippage of the RdRp to occur as, surprisingly, a small number of additional G residues were inserted into the F mRNA in MuVJL2-infected cells at the GG sequence after the first motif (Fig. 3b). This process resembles slippage of the viral polymerase at the P/V editing site in the P gene. The F gene...
of the Kilham strain of MuV (MuVKH) is similar to MuVJL2 in that it contains tracts of both six and seven adenosine residues at the end of the F gene (Tecle et al., 2000; Lemon et al., 2007), but is similar to MuVJL5 in that it contains GA instead of GG between the two tracts (Fig. 2). When the 3′-terminal region of the MuVKH F mRNA was sequenced, there was no oligo-G insertion, which suggests that the presence of the second G of the GG between the two adenosine tracts is necessary for the oligo-G insertion event to occur (Fig. 3e).

Development of a reverse-genetics system for MuVJL2

It became clear that not only was the sequence of our MuVJL2 component (Afzal et al., 1993) (received as an early passage after isolation and plaque purification of the MuVIL5 and MuVJL2) different from the MuVIL5 sequence, but also that the level of variation between our MuVJL2 sequence and the published MuVJL2 sequence was significant (51 nt). In order to facilitate further molecular studies, to investigate the phenotypes of recombinants between MuVJL2 and MuVJL5, and other MuV strains, and to where a potential minor mutation event was detected in the P gene (three nucleotides, 2459, 2534 and 2582, are all T in the full-length plasmid, but C in most of the P gene clones), because both MuVJL5 and the previously published MuVJL2 sequence have T at these three positions. Restriction-enzyme sites at intergenic locations between genes encoding the envelope proteins of pMuVJL5 have been incorporated during the construction of pMuVJL2 (the unique restriction site between F and SH is, however, AvrII in pMuVJL2 rather than BmgBI as in pMuVJL5, because pMuVJL2 has an additional BmgBI site in the L gene and there are both SgfI and SapI unique restriction sites between SH and HN in pMuVJL5). Additional unique restriction-enzyme sites have been generated between all other genes and internal to all viral genes (except for that of SH) and spaced fairly evenly throughout the L gene to facilitate molecular studies (Fig. 4).

Although helper plasmids appear to be interchangeable in the rescue systems for all molecular clones tested, from our direct experience, it appeared advisable to use homologous plasmids in order to avoid inter-strain recombination events driven by vaccinia virus (data not shown). Therefore, MuVIL2-specific helper plasmids encoding the N, P and L genes (pCG-NIL2, pCG-PIL2 and pCG-LIL2),
respectively) were constructed in the CMV promoter-driven vector pCG and these were used successfully in the rescue of the pMuVJL2. A mumps minigenome construct that encodes enhanced green fluorescent protein (EGFP) (pMuVEGFP) has been constructed by replacing the chloramphenicol acetyltransferase open reading frame of pMuVDICAT (Clarke et al., 2000) with that for EGFP. pMuVEGFP has been rescued with both the MuVJL2 and MuVJL5 sets of helper plasmids (data not shown). A recombinant virus, rMuVJL2, was rescued successfully from plasmid pMuVJL2. The growth of this virus was very similar to that of the original plaque-purified virus MuV JL2, confirming that the synonymous genetic alterations that we have made do not affect virus growth dynamics in Vero cells (Fig. 5).

**Construction of chimaeric rMuVJL2 and rMuVJL5 viruses to characterize the oligo-G insertion at the end of the F mRNA of MuVJL2**

In order to determine whether the oligo-G insertion at the end of the F mRNA of MuVJL2 was determined by the nucleotide sequence at the end of the MuVJL2 F gene, by characteristics of the viral polymerase of strain MuVJL2 or by other determinants in the genome of this MuV strain, the F gene of MuVJL2 was transferred into MuVJL5 and the F gene of MuVJL5 into MuVJL2 by using the unique between-gene restriction sites that are present in the plasmids. The corresponding viruses were rescued and the 3' termini of the F mRNAs were characterized. The recombinant rMuVJL5 (FJL2) displayed the same oligo-G insertion as the MuVJL2 F gene, and the rMuVJL2 (FJL5) displayed no oligo-G insertion (Fig. 6). This indicates that the oligo-G insertion was associated with the F gene sequence of MuVJL2 rather than being a characteristic of the viral polymerase of strain MuVJL2.

**DISCUSSION**

The origin of the sequence variation between the MuVJL5 and MuVJL2 components of the live-attenuated JL vaccine

![Figure 4](image-url)  
**Fig. 4.** Molecular clone of MuV JL2, indicating gene boundaries and restriction sites in pMuVJL2. The bar shows the antigenome of pMuVJL2 and the locations of viral genes (not to scale). Arrows beneath the bar indicate the location of unique restriction sites suitable for ligase-independent cloning using exonuclease III in pMuVJL2. The vector sequence flanking the antigenome contains a NcoI site upstream of a T7 RNA polymerase promoter located 5' to the antigenome (i.e. to the left of N) and a KasI site downstream of the antigenome 3' terminus (i.e. to the right of L) which is internal to the hepatitis delta ribozyme (these restriction sites are shown in bold). (a) Restriction sites present in the consensus MuVJL2 sequence – these were either already unique in the consensus MuVJL2 sequence or made unique by mutagenesis of sites at other locations in the MuV genome or the plasmid vector. (b) Restriction sites introduced into the final clone by in vitro mutagenesis. Additional Smal, AvrII, BsrGI and XhoI restriction sites in the MuVJL2 sequence (c) were removed by in vitro mutagenesis. A SapI site and two FspI sites were removed from the vector sequence by in vitro mutagenesis or deletion to render sites in the MuVJL2 sequence unique in the final clone. Restriction-enzyme names are abbreviated for clarity. Details of their position in the MuVJL2 sequence are available on request. The asterisks indicate that these sites are unique in the plasmid DNA which is methylated, as there are two sites at 11408–11413 and 11608–11613 that are also cleavable with Stul and NruI, respectively, in unmethylated plasmid DNA.

![Figure 5](image-url)  
**Fig. 5.** Growth curves of MuVJL2 and rMuVJL2. Supernatant (a) and cell-associated (b) titres of samples harvested from infected Vero cells at days 0–7 post-infection were determined by TCID50. The stock plaque-purified MuVJL2 supplied by Dr M. Afzal and the final recombinant virus rescued from cDNA are shown as MuVJL2 (■) and rMuVJL2 (▲), respectively. Error bars represent SD.
is unclear. The JL vaccine was derived from a single clinical isolate. These, in general, display a consensus sequence with little detectable variation and, hence, it is likely that some part of the variation between the two vaccine components has been generated after the original isolation and during the attenuation process. Selection of a more neurovirulent variant of MuVJL5 has been shown to be associated with only three amino acid changes and relative sequence stability of this virus (Rubin et al., 2003). The substantial sequence differences between MuVJL2 determined in different laboratories suggest that cytosine or adenine deamination events may play a significant role in the generation of diversity between the MuVJL strains. It is to be noted that Amexis et al. (2002) did not succeed in propagating a MuVJL2 virus, but that the sequence was derived from sequence variations found in the vaccine. Though it was inferred to be preferably propagated in chicken embryo cells, their MuVJL2 could not be propagated on Vero cells and, hence, they suggested that MuVJL2 was a not completely defective satellite virus. Our sequence is derived from a plaque-purified Vero cell-propagated MuVJL2 isolate. Amexis et al. (2002) posed the question of whether the MuVJL2 sequence is a passenger virus of MuVJL5. The fact that we were able to set up a rescue system based on the MuVJL2 consensus sequence with its own helper plasmids indicates that the vaccine is a mixture of two independently replicating viruses. In fact, titres of MuVJL2 are routinely at least 1 log10 higher than those of MuVJL5 in Vero cells in our hands. This, and the number and location of unique restriction-enzyme sites that did not affect the viral protein sequences and the even spacing of unique restriction sites in pMuVJL2, should be of benefit in future molecular studies of MuV and may prove to be advantageous in the use of the MuVJL2 rescue system. The prevalence of C→U and U→C mutations and the identification of the localized nature of the biased hypermutation indicate that deamination reactions by ADAR- or APOBEC-like enzymes may play a role in the generation of the sequence variation between MuVJL5 and MuVJL2, although this remains to be formally proven. In most cases, biased hypermutation events lead to functional impairment of the affected region of the viral genome, for example in measles virus sequences obtained from cases of subacute sclerosing panencephalitis (Cattaneo et al., 1988). Interestingly, loss of function does not seem to be the case here. The potential events in L and N genes are located in the 3′ non-coding regions. One potential event in the P gene affects an area with low sequence similarity at the N-terminal part of the V/P protein. The other event present in most but not all of our MuVJL2 P clones affects the V but not the P protein and this may alter the interferon sensitivity of the MuVJL2 virus. Biased hypermutation has been described for several other viruses (Bass, 2002) and may reflect the deaminating activity of the ADAR or APOBEC enzymes themselves (Bass, 2002; Bishop et al., 2004) or their ability to bind to RNA (Nie et al., 2007).

This study shows for the first time, to our knowledge, the MuV genome and 5′ termini of the mRNAs and that these had been predicted correctly from the conservation of motifs, and demonstrates the capped nature of these mRNAs in contrast to the uncapped terminal sequences of the genome. Earlier studies were not able to determine the precise mRNA start sites, although polyadenylation signals had been identified correctly (Elango et al., 1988). The polyadenylation signal at the end of the F gene of MuVJL2 required clarification because of the sequence variation amongst strains of MuV at this point. Read-through from the F to SH transcription units is found in MuVEnders

---

Fig. 6. 3′-Terminal sequences of F genes and transcripts of rMuVJL2(FJL5) and rMuVJL5(FJL5). (a, b) Sequence from rMuVJL2(FJL5); (c, d) sequence from rMuVJL5(FJL5). (a) and (c) show sequence electrochromatograms of the virion sequence of the F polyadenylation region cut to the first nucleotide difference between MuVJL5 and MuVJL2 in the SH gene; (b) and (d) show the 3′-terminal region of the F mRNA. 2. rMuVJL2(FJL5) has the F sequence characteristic of rMuVJL5 and the SH sequence characteristic of rMuVJL2, and rMuVJL5(FJL5) has the F sequence characteristic of rMuVJL2 and the SH sequence characteristic of rMuVJL5. The oligo-G insertion event in (d) is indicated by a black bar and polyadenylation in (b) and (d) by a dotted arrow.
In conclusion, we have demonstrated significant sequence variation and instability in the MuV\textsuperscript{JL2} component of the JL vaccine. In part, these sequence changes may be generated by activity of cytosine and/or adenine deaminases in the cell. However, the inter-relationship of MuV\textsuperscript{JL2} and MuV\textsuperscript{JL5} is not clear and the sequence diversity even between isolates of MuV\textsuperscript{JL2} raises further questions about the relationships between the two vaccine components. We have developed a versatile rescue system for MuV based on MuV\textsuperscript{JL2} and used this and the MuV\textsuperscript{JL5} rescue system to prove that an oligo-G insertion event at the end of the F gene of MuV\textsuperscript{JL2} is determined by the sequence at the slippage site.

ACKNOWLEDGEMENTS

This work was supported by Wellcome Trust grant no. 064263. We thank Dr D. Clarke for the supplying the MuV\textsuperscript{JL2} rescue system, Dr K. Lemon in our laboratory for many plasmids, oligonucleotides and discussions and Ms Rosie Neeson and Ms Caitriona Byrne for technical assistance.

REFERENCES


