Molecular cloning, physical mapping and cross-hybridization of the murine adenovirus type 1 and type 2 genomes

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Murine adenovirus (MAd) type 1 strain FL and type 2 strain K87 genomes were cloned into plasmid pAT153 as HindIII restriction fragments. The MAd-1 and MAd-2 DNA genomes, 30.10 kb and 34.71 kb in length respectively, were mapped using BglII, ClaI, EcoRI, HindIII and SphI restriction endonuclease cleavage sites. In view of the large differences found between the MAd-1 and MAd-2 genomes in terms of the number and location of restriction sites, cross-hybridization experiments were performed. Homologous DNA sequences were located on the MAd-1 and MAd-2 physical maps. Both viruses are also genetically related to human adenovirus type 2 (HAd-2). Nucleotide sequences shared by HAd-2 and the MAds code for structural proteins, which may explain the antigenic similarities between these viruses from different origins. Our results confirm the existence of two distinct adenovirus species in the mouse.

Introduction

Two related but distinct adenoviruses have been isolated from the mouse. The FL strain of murine adenovirus (MAd) type 1 was originally found in a contaminated Friend leukaemia virus stock (Hartley & Rowe, 1960), whereas the K87 strain of MAd-2 originates from the faeces of clinically normal mice (Hashimoto et al., 1966). MAd-1 produces a fatal generalized infection only in newborn mice (Blailock et al., 1968; Hartley & Rowe, 1960), whereas MAd-2 is associated with diarrhoeal disease in both neonatal and adult mice (Sugiyama et al., 1967). The two MAds differ in their pathology and tissue range but share common antigenic properties, as shown by neutralization, haemagglutination-inhibition and complement-fixation tests (Lussier et al., 1987; Smith et al., 1986; Van der Veen & Mes, 1974; Wigand et al., 1977). Because of this serological cross-reactivity, MAd-2 is still considered in the literature to be a variant of MAd-1 (Matthews, 1982; Wigand et al., 1982).

Restriction endonuclease analysis was recently used successfully to demonstrate the distinctiveness of the MAd types 1 and 2 genomes (Hamelin et al., 1988). A physical map has until now only been constructed for MAd-1 DNA (Larsen & Nathans, 1977; Larsen et al., 1979). Cross-hybridization tests with a series of restriction fragments of MAd-1 DNA and human adenovirus type 2 (HAd-2) DNA revealed a degree of genetic similarity between the two viruses (Larsen et al., 1979). Similar experiments have not yet been performed with MAd-2 DNA.

Here, we report a restriction map for MAd-2 DNA obtained with the use of six restriction endonucleases, and we compare the number and location of these restriction sites with those on the MAd-1 genome. In addition, our experiments allowed us to delimit homologous nucleotide sequences between the MAds and HAd-2.

Methods

Viruses. The MAd-1 FL and MAd-2 K87 strains were propagated in mouse CMT-93 cells as previously described (Hamelin et al., 1988).

DNA preparations. Virus DNA was extracted from MAd-infected mouse cells by the SDS-Pronase-phenol method described by Hamelin et al. (1988), and was purified by isopycnic centrifugation in caesium trifluoroacetate (Pharmacia) with ethidium bromide. Plasmid DNA was prepared for the restriction enzyme digestions and cloning experiments using the cleared-lysate technique (Dion et al., 1985) and precipitation with 0.5 M-NaCl and 5% polyethylene glycol 8000 (Union Carbide). The restriction endonucleases BglII, ClaI, EcoRI, HindIII and SphI and T4 DNA ligase (Gibco) were used according to the manufacturer's instructions.

Cloning. Random HindIII subgenomic fragments of MAd DNA were cloned into the plasmid pAT153 according to standard procedures (Hamelin et al., 1984).

Electrophoresis. Agarose (1%) gels were prepared and electrophoresis was performed in buffer (0.1 M-Tris–borate, and 2 mM-EDTA pH 8.3) at 50 V for 16 h (or at 90 V for 2 h for minigels). Separation of DNA fragments was also carried out by pulsed-field gel electrophoresis, at 50 V for 24 h (0.50 s forward, 0.25 s reverse) according to Larson et al. (1987). The buffer concentration was reduced by half for this method. Fragments were visualized with a 254 nm ultraviolet trans-illuminator after being stained with ethidium bromide.

Mapping. Cloned HindIII fragments were cleaved with BglII, ClaI, EcoRI, HindIII and SphI, and ordered by examining their overlapping regions after single and multiple digestions (Fitch et al., 1983).
Fig. 1. Electrophoretic fragment patterns of MAd-1 strain FL (lane 1) and MAd-2 strain K87 (lane 2) DNA molecules after cleavage with four restriction enzymes. Digestion products were separated by electrophoresis in a standard 1% agarose gel at 50 V for 16 h.

Fig. 2. The MAd-1 (a) and MAd-2 (b) subgenomic HindIII restriction fragments cloned into plasmid pA153. MAd-1 fragments are 12.22 kb (A), 7.50 kb (B), 3.67 kb (C), 3.05 kb (D), 2.50 kb (E), and 1.20 kb (F) in size. The corresponding MAd-2 DNA fragments are 14.45 kb (A), 10.46 kb (B), 8.08 kb (C), and 2.13 kb (D) in size. A 1 kb ladder from Gibco (lanes 1) and the viral genome cleaved with HindIII (lanes 2) were used to identify each of the cloned fragments. Electrophoresis was carried out in 1% agarose minigels at 90 V for 2 h.

Fig. 3. Migration of BglII fragments of the MAd-1 and MAd-2 genomes in 1% agarose minigels under standard (a and b) or pulsed-field (c and d) electrophoresis conditions. Incompletely digested MAd-1 or MAd-2 DNA molecules with a length greater than 12 kb were better separated by the latter technique. Linkage of restriction fragments was determined after digestion of approximately 0.2 μg of MAd-1 or MAd-2 DNA with 2 units of BglII for 1, 2, 4, 8, 16 and 32 min (lanes 2 to 8 respectively). High M. DNA markers (lanes 1) and a 1 kb ladder from Gibco (lanes 2) were used as references.

**DNA hybridizations.** MAd or HAd DNA fragments in agarose gels were transferred onto Magna nylon membranes (MSI) by electroblotting (Pramatarova et al., 1992). The membranes were blotted using Whatman 3MM filters saturated with 0.5 M NaOH and 1.5 M NaCl for 5 min, transferred to a dry 3MM filter for 5 min, placed on another 3MM filter saturated with 10 mM-Tris-HCl pH 8.0 and 1.5 M NaCl for 5 min, and baked at 120 °C for 30 min. DNA probes were labelled with biotin-7-dATP using a nick translation system (Gibco). The probe target hybrids were detected with a BluGENE non-radioactive nucleic
acid detection system (Gibco). All procedures were carried out according to the manufacturer’s instructions. Colour development was allowed to proceed for 16 h in the dark.

**Results**

MAd-1 FL and MAd-2 K87 have long been thought to belong to the same adenovirus species. However large differences at the genomic level were observed when the restriction profiles of these two viruses were compared (Fig. 1). To distinguish between them further, we determined the position of the *BglII, ClaI, EcoRI, HindIII* and *SphI* restriction sites on the MAd-1 and MAd-2 genomes. Each genome was cloned into plasmid pAT153 as *HindIII* fragments (Fig. 2). Problems related to the presence of protein at the termini of adenovirus DNA molecules (Kelly, 1984; Sussenbach, 1984; Darai et al., 1985) were apparently solved by the Pronase digestion step in the extraction procedure.

MAd-1 DNA is cleaved at five different sites by *HindIII* to give fragments of 12.22, 7.50, 3.67, 3.05, 2.50 and 1.20 kb (Table 1). MAd-2 DNA digested with this enzyme gives four restriction fragments with lengths of 14.45, 10.06, 8.08 and 2.13 kb. Cloned fragments therefore totalled 30.14 kb (MAd-1) and 34.71 kb (MAd-2). Similar sizes have already been reported for other

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**Fig. 4.** Maps of the cleavage sites of five different restriction endonucleases in MAd-1 strain FL (a) and MAd-2 strain K87 (b) DNAs. The fragments are named alphabetically in order of decreasing size. Map unit and length scales are given below each map.

**Fig. 5.** Cross-hybridization of the MAd-1 and MAd-2 genomes. (a) MAd-1 DNA fragments produced by *BglII* (lane 2), *ClaI* (lane 3), *EcoRI* (lane 4), *HindIII* (lane 5) and *SphI* (lane 6) and stained with ethidium bromide. (b) MAd-1 DNA fragments detected by a biotin-labelled MAd-2 DNA probe after electroblotting onto a nylon membrane. Hybridization was carried out at 68 °C for 16 h. Lanes 1 and 7 contain a 1 kb DNA ladder.
adenoviruses (Gingeras et al., 1982; Jouvenne et al., 1987; Matthews, 1982; Wigand et al., 1982). It is interesting to note that the MAd-1 genome contains about 4-5 kbp less than MAd-2.

To elaborate rapidly on the physical maps of the MAd-1 and MAd-2 genomes, electrophoresis in agarose minigels was used (Fig. 2). To separate large DNA fragments, we used pulsed-field electrophoresis in agarose minigels. This technique was particularly useful for separating the large restriction fragments that arose with partial digestion of DNA (Fig. 3). To analyse small fragments resulting from a double or triple enzymatic digestion, polyacrylamide gels were used (Bellomy & Record, 1989). Using these different electrophoresis techniques, we have been able to determine exactly where restriction endonucleases BglII, ClaI, EcoRI, HindIII and SphI cleave the MAd-1 and MAd-2 DNAs (Fig. 4).

Physical mapping of the MAd-1 genome with the ClaI and SphI enzymes is reported here for the first time, and other data are used as references. All the results obtained using MAd-2 are novel. The structural organization of the MAd-1 genome appears quite different from that of the MAd-2 genome when analysed by restriction enzyme digests.

Next, nucleotide sequence similarities between the two MAds were investigated. For this purpose, MAd-2 DNA labelled with biotin was hybridized to MAd-1 DNA cleaved with BglII, ClaI, EcoRI, HindIII and SphI, on nitrocellulose filters. The results showed that MAd-1 and
MAd-2 were genetically related to an extent (Fig. 5). The labelled MAd-2 DNA probe hybridized to several large MAd-1 restriction fragments, including the HindIII A and the SphI A fragments. These overlap by about 6000 bp in the MAd-1 DNA molecule (map units 0-5 to 0-7; Fig. 4).

According to Larsen et al. (1979), MAd-1 shows a degree of homology with HAd-2, in the nucleotide sequences of genes that code for structural proteins. The results shown in Fig. 6 and 7, (a) and (b) confirm this observation. MAd-2 and HAd-2 DNA molecules were then cross-hybridized in order to find similar regions of homology (Fig. 6 and 7, c and d), and were also shown to have nucleotide sequences in common (Fig. 8). The two regions of the HAd-2 genome that were found to be homologous to regions of MAd-2 DNA were in similar positions to the regions homologous to MAd-1 DNA (Fig. 9).

**Discussion**

Restriction endonuclease analysis was recently used to differentiate between MAd-1 FL and MAd-2 K87 (Hamelin et al., 1988). DNA obtained from these two viruses was cleaved with numerous restriction enzymes and in each case the MAd genomes appeared to be different, both in the number and the length of the fragments observed after agarose gel electrophoresis (Fig. 1). Despite a degree of antigenic relatedness, the FL and K87 strains were shown to represent two distinct
Fig. 9. Regions of the HAd-2 genome that hybridize to MAd-1 and MAd-2 DNA probes (boxed). Shaded areas show HAd-2 DNA sequences that are homologous to both MAd-1 and MAd-2. The map is labelled as in Fig. 4.

Table 1. Size of MAd-1 and MAd-2 DNA restriction fragments (kb)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Fragment</th>
<th>BgIII</th>
<th>ClaI</th>
<th>EcoRI</th>
<th>HindIII</th>
<th>SphI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAd-1 A</td>
<td>16.94</td>
<td>15.59</td>
<td>21.34</td>
<td>12.22</td>
<td>9.75</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8.25</td>
<td>14.55</td>
<td>6.40</td>
<td>7.50</td>
<td>8.20</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.10</td>
<td>2.40</td>
<td>3.67</td>
<td>5.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.85</td>
<td>3.05</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2.80</td>
<td>1.63</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.20</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| MAd-2 A | 9.35     | 11.03 | 21.94| 14.45 | 18.34   |
| B       | 6.78     | 10.00 | 6.52 | 10.06 | 9.74    |
| C       | 6.18     | 7.68  | 3.64 | 8.08  | 4.44    |
| D       | 4.20     | 3.16  | 1.80 | 2.13  | 2.20    |
| E       | 2.93     | 1.40  | 0.82 |       |         |
| F       | 2.05     | 0.73  |      |       |         |
| G       | 0.91     | 0.72  |      |       |         |
| H       | 0.83     |       |      |       |         |
| I       | 0.61     |       |      |       |         |
| J       | 0.55     |       |      |       |         |
| K       | 0.33     |       |      |       |         |

species of MAd. The physical maps of the MAd genomes were expected to be different and the level of sequence homology to be relatively low, because of the dissimilarities in the migration of DNA fragments from each MAd during restriction analysis.

According to Larsen & Nathans (1977), the MAd-1 DNA molecule is about 30-3 kbp in length. By totalling the lengths of all of the MAd-1 DNA fragments obtained after cleavage with the restriction enzymes BgIII, ClaI, EcoRI, HindIII and SphI we obtained a value of 30-14 kbp. The MAd-2 genome, measured here for the first time, was found to be 34-71 kbp in length. In general, adenovirus DNA molecules are between 30 and 60 kbp in length (Gingeras et al., 1982; Jouvenne et al., 1987; Matthews, 1982; Wigand et al., 1982). MAd-1 and MAd-2 could thus be considered as typical adenoviruses.

Physical maps of the MAd-1 and MAd-2 genomes were constructed with five different restriction enzymes (Fig. 4). The positions of BgIII, EcoRI and HindIII restriction sites on the MAd-1 DNA molecule, as determined by Larsen & Nathans (1977), was confirmed. Results obtained with ClaI and SphI complete the physical mapping of the MAd-1 genome. MAd-2 is mapped here for the first time (Fig. 4). Large differences were observed in the number and position of the restriction sites of the MAd-1 and MAd-2 DNA molecules with all five enzymes. This implies that the two MAds are distinct adenovirus species.

An estimate of the extent of homology between DNA sequences can be made by comparisons of the changes in restriction endonuclease cleavage sites (Engels, 1981; Gotoh et al., 1979; Upholt, 1977). The MAd-1 and MAd-2 restriction maps are very different and the viruses were thus expected to show little sequence similarity. The observation that the overlapping MAd-1 HindIII A and SphI A fragments reacted with the total MAd-2 DNA probe in cross-hybridization experiments indicates that the two viruses have about 20% of their nucleotide sequences in common. This is in sharp contrast to the type 1 and type 2 canine adenoviruses, which share at least 57% of their nucleotide sequences (Jouvenne et al., 1987).

MAd-1 DNA is shorter than HAd-2 DNA (30-10 kbp compared with 35-94 kbp) but the two viral genomes can be aligned, based on the map position of two homologous DNA segments discovered by Larsen et al. (1979). Regions of homology are found between map positions 0-12 and 0-18 and between 0-53 and 0-62 of the HAd-2 DNA sequence. Similar regions are found between coordinates 0-06 and 0-16 and between 0-54 and 0-67 in the MAd-1 DNA map. MAd-2 DNA used as a probe recognized two regions of homology, from 0-17 to 0-25 and from 0-51 to 0-59 map units, on the HAd-2 genome (Fig. 6). Sequences between 0-18 and 0-22 and between 0-57 and 0-71 map units were recognized on the MAd-2 genome by the HAd-2 probe. The sequences shared by MAd-2 and HAd-2 partly overlap those shared by MAd-1 and HAd-2 (Fig. 9). The DNA regions recognized by the MAd-1 probe in HAd-2 contain genes coding for structural components of the virus (Ball et al., 1988, 1989; Beard et al., 1990; Gingeras et al., 1982; Larsen et al., 1979; Raviprakash et al., 1989). The observation that a MAd-2 probe recognizes approximately the same sequences as the MAd-1 probe in HAd-2 DNA suggests that the two MAds have genes encoding similar hexon and structural proteins. Cross-reactivity between MAd-1 and MAd-2 at the immunological level may thus be, at least in part, due to these gene products. Differences in their pathogenicity and tissue range may be related to the large differences already observed at the sequence level by restriction endonuclease analysis and DNA hybridization between the two MAd genomes.
The present results are important if MAd-1 and MAd-2 are to be considered either as models for studying adenovirus pathogenesis or as potential expression vectors in mouse cells.

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References


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