Isolation of Transformation-Defective, Replication-Nondefective Early Region 1B Mutants of Adenovirus 12

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We isolated three adenovirus 12 early region 1B mutants (in205B, in205C, and di205) by ligation of the cleaved DNA-protein complex and transfection of human embryo kidney cells with the ligation products. These mutants could replicate efficiently in human embryo kidney or KB cells but showed markedly reduced transforming capacities both in vitro and in vivo. In cells infected with the mutants, the early region 1B gene was transcribed efficiently. In cells infected with in205B, the products corresponding to the early region 1B-coded 19,000-molecular-weight polypeptide was detected by in vitro translation but not immunoprecipitated extract of labeled cells. In cells infected with in205C or di205, the products corresponding to the same polypeptide were not detected by either in vitro translation or immunoprecipitation of labeled cell extracts. The results suggest that the 19,000-molecular-weight polypeptide encoded by early region 1B is required for cell transformation but not for viral propagation.

There have been many studies on early region 1B (E1B) gene functions of human adenoviruses. Some of the adenovirus 2 or 5 (Ad2 or Ad5) mutants have been isolated as host range mutants with mutations on the E1B gene (11, 13, 26). Most of these mutants can grow in 293 cells, Ad5-transformed human embryo kidney (HEK) cells (9), but fail to grow in HELa, HEK, or KB cells. Although some host range mutants can grow in HEK cells (11), the results indicate that E1B gene products are required for the growth of viruses. The mutants also show much reduced capacities for transforming rodent cells (13, 23).

When rodent cells are transfected with DNA fragments encompassing both E1A and E1B genes, the cells are transformed completely (8, 27, 28). However, DNA fragments encompassing only the E1A gene transform cells only incompletely or partially (12, 24). It is deduced from these observations that E1B gene products play an important role in the transformation of cells. DNA sequence data of the E1B gene reveal that there are at least two polypeptides encoded by E1B (19,000-molecular-weight [19K] and 54K polypeptides in Ad12) (3, 4, 16, 25, 29). The smallest Ad12 DNA fragment capable of transforming rodent cells completely is the HindIII-G (0 to 6.8 map units) (22). This fragment encompasses the whole E1A, the whole coding region for the 19K polypeptide, and the left-hand part of the coding region for the 54K polypeptide (3, 16, 25). This observation suggests that the 19K polypeptide is required in the complete transformation of cells.

In this paper, we describe the isolation of Ad12 mutants with insertions or a deletion in the E1B-19K polypeptide-coding sequence of Ad12 and properties of the mutants for productive infection and cell transformation. The results show that these mutants have much reduced transforming capacities but are not defective in productive infection and suggest that the 19K polypeptide is required for cell transformation and not productive infection.

MATERIALS AND METHODS

Cells and viruses. KB and HEK cells were used for the propagation of viruses. A rat cell line 3Y1, established from a Fischer rat embryo (15), was used for the transformation of cells. Cells were cultured in Eagle minimal essential medium (MEM) supplemented with 10% fetal calf serum. The prototype strain of Ad12 was used. As a source of the Ad12 E1B gene, plasmid pASC, which contains the Ad12 E1A and E1B sequence (0 to 10.2 map units), was used (6). Plasmid pASC was propagated in Escherichia coli HB101.

Preparation of Ad12 DNA-protein complex. Ad12 DNA-protein complex was prepared as described by Dunsworth-Browne et al. (5).

Enzymes and reaction conditions. Restriction endonucleases were purchased from Boehringer Mannheim Corp., Bethesda Research Laboratories, New England Biolabs, and Takara Shuzo. The conditions for DNA digestion were as specified by the suppliers. The conversion of cohesive ends of DNA fragments to flush ends was carried out with the large fragment of E. coli DNA polymerase I (Klenow's enzyme). For reactions, DNA fragments were dissolved in 10 μl of buffer containing 50 mM Tris-hydrochloride (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, and 150 μM nucleotide triphosphate. To this solution, 1 U of enzyme was added. After incubation for 15 min at room temperature, the reaction was terminated by heating at 70°C for 10 min. For ligation, DNA fragments were dissolved in buffer containing 50 mM Tris-hydrochloride (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, and 1 mM ATP. Then T4 DNA ligase was added (final solution, 2 to 3 U/ml) to the mixture, which was incubated at 4 or 12°C for 3 h.

DNA sequence analysis. The mutated parts of the virus genomes (1.7 to 6.8 map units) were cloned in pBR322. The 5′-end-labeled DNA fragments were obtained by using the HaeIII sites (1.707-base-pair [bp] position, in205B and in205C) or PvuII site (1.904-bp position; di205) located near the mutations, and the DNA sequences were analyzed as described by Maxam and Gilbert (18), except that 4% formic acid was used in place of 4% pyridinium formate for the adenosine plus guanine reaction.

Transfection of cells with DNA. This was carried out by the calcium phosphate technique as described by Graham and van der Eb (10).

Nuclease S1 mapping of mRNA. The procedures were as described previously (1, 21). Briefly, cytoplasmic RNA was extracted from cells and hybridized with an excess amount
of 32P-labeled Ad12 DNA in hybridization buffer containing 80% formamide, 0.4 M NaCl, 0.04 M PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid) (pH 6.4), and 1 mM EDTA at 52°C for 3 h. The mixture was treated with single-strand-specific nuclease S1, and the S1-resistant DNA-RNA hybrids were analyzed by electrophoresis in a 1.4% agarose gel with alkaline buffer.

In vitro translation of mRNA. mRNAs complementary to E1A and E1B genes were selected by hybridization with DNA probes as described by Ricciardi et al. (20). Briefly, 1 μl of Ad12 HindIII-G was immobilized on a nitrocellulose filter, and 350 μg of cytoplasmic RNA extracted from Ad12-infected KB cells at 14 h postinfection (p.i.) was hybridized to the DNA probe in the hybridization buffer at 45°C for 2 h. The filter was washed 10 times with SSC (0.15 M NaCl plus 0.015 M sodium citrate) and 0.5% sodium dodecyl sulfate at 55°C and twice with buffer containing 2 mM EDTA (pH 7.9) at 55°C. Then the filter was soaked in distilled water, heated at 100°C for 1 min, and cooled quickly. The filter was removed, and the eluted RNA was precipitated with ethanol after the addition of 20 μg of E. coli tRNA as a carrier. Under this condition, mRNAs complementary to E1A and E1B genes are selected efficiently. These mRNAs were translated in nucleic-acid-treated reticulocyte lysate containing [35S]methionine (New England Nuclear Corp., Boston, Mass.). The conditions were as specified by the supplier. The products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17) or by two-dimensional gel electrophoresis (19), and bands or spots of the products were detected by fluorography (2).

Immunoprecipitation. KB cells were infected with Ad12 at 10 PFU per cell. After the medium was replaced with methionine-free Eagle MEM at 12 h p.i., the cells were labeled with [35S]methionine (200 μCi/ml) from 14 to 16 h p.i. Cells were harvested and suspended in 100 μl of RIPA buffer (10 mM Tris-hydrochloride [pH 7.5], 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 150 μM phenylmethylsulfonyl fluoride, 150 mM NaCl). The cell suspension was sonicated, kept at 4°C for 10 h, and centrifuged to remove the cell debris. A sample of anti-19K sera (sera from rats bearing Ad12 tumors) was added to the cell extract, and the mixture was incubated at 4°C for 4 h. A sample of the 20% suspension of formaldehyde-fixed Staphylococcus spp. (protein A [14]) was added to the mixture, which was incubated at 4°C for 30 min. The staphylococcal cells were collected by centrifugation and washed three times with RIPA buffer. The lysis buffer (9.5 M urea, 2% Nonidet P-40, 2% Ampholine) (19) was added to the cells and heated at 68°C for 10 min. The cells were removed by centrifugation, and the supernatant was analyzed by two-dimensional gel electrophoresis (19).

Transformation assay. The transformation assay was performed as described by Sjömo et al. (30). Monolayer cultures of rat 3Y1 cells were infected with viruses at 10 PFU per cell and subcultured at 10^4 cells per 5-cm dish. After 3 days, the medium was replaced with low calcium (0.1 mM Ca^2+ ) medium. Medium was changed every 3 days thereafter. Cells were fixed with 10% Formalin solution and were stained with Giemsa 4 weeks after infection.

Colonies were scored on soft agar. Cells in a small dish were dispersed in phosphate-buffered saline containing 0.02% EDTA and collected by centrifugation. Then 10^3 cells were suspended in 4 ml of 0.4% agar medium (Eagle MEM, 0.1 mM Ca^2+, 10% fetal calf serum) and layered on the base layer of 0.8% agar with the same composition in a 5-cm dish. They were cultured for 3 weeks at 37°C.

Induction of tumors in hamsters. The virus suspension was injected subcutaneously into baby hamsters within 24 h after birth. The hamsters were observed for 90 days.

RESULTS

Isolation of Ad12 E1B mutants. The strategy for the isolation of Ad12 E1B mutants is outlined in Fig. 1. Since restriction enzyme BstEII cleaves Ad12 DNA at a single site (10.2 map units), the Ad12 DNA-protein complex (DNA with terminal protein) was cleaved with BstEII to produce small left-terminal and large right-terminal portions. Plasmid pASC, containing Ad12 E1A and a portion of E1B (0 to 10.2 map units), was mutagenized by insertion or deletion in the E1B sequence as described below. Cleaved DNA-protein complex and mutagenized plasmids were mixed and ligated. HEK cells were transfected with the ligated DNA-protein complex. After forming plaques in HEK cells, clones showing restriction cleavage patterns for insertion or deletion were selected.

The mutant plasmid DNAs were constructed as shown in Fig. 2. Since the AccI site at 4.5 map units is located on the coding sequence for the E1B-19K polypeptide, we introduced mutations at this site. Plasmid pASC was modified to produce pIN5B (insertion of 12 bp), pIN5C (insertion of 16 bp), and pDL5-6 (deletion of 175 bp).

These mutated plasmids (pIN5B, pIN5C, and pDL5-6) were purified and amplified in E. coli. After cleavage with BstEII, the plasmid DNA fragments were isolated by agarose gel electrophoresis and electroelution. Each of the mutated fragments was mixed with the Ad12 DNA-protein complex digested with BstEII at 37°C, and the mixtures were treated with T4 DNA ligase. HEK cells were transfected with each of the ligation products and kept under agar overlay. After incubation for 3 weeks, plaques became visible. Virus clones were isolated from plaques and propagated in KB cells. Restriction enzyme cleavage patterns of
viral DNA from each clone were examined by electrophoresis. The virus clones which contained expected insertions or deletions were selected and established as mutants after plaque purification in HEK cells (in205B from pINSB, in205C from pINSC, and dl205 from pDL5-6). For instance, transfection of HEK cells with ca. 50 μg of pINSC DNA ligated with ca. 50 ng of Ad12 DNA-protein complex resulted in the formation of six plaques. Of these plaques, one consisted of a mutant, one of a wild-type (WT) virus, and the remaining four of the mixture. Mutants were named as by the method of Ginsberg et al. (7).

Nature of mutants. The restriction enzyme cleavage patterns of DNAs from mutants are shown in Fig. 3. In Ad12 WT DNA, BamHI-A (8,020 bp; 0 to 23.4 map units) was located at the left end of the genome. Cleavage with BamHI showed that new BamHI sites were introduced in in205B and dl205 DNAs (Fig. 3, lanes b and d). Since in205B DNA was formed after the addition of BamHI linkers, the presence of the BamHI site was rational. As for dl205 DNA, since the ligation of BamHI site and Sau3AI site in in205B regenerated the BamHI site as deduced from the sequence date (3, 25), the presence of a new BamHI site was also rational. Cleavage with Clai showed that a new Clai site was introduced instead of the BamHI site in in205C DNA (Fig. 3, lane g). In dl205 DNA, the HindIII-G (2,320 bp; 0 to 6.8 map units) is a little shorter than that of WT DNA because of the deletion of 175 bp (Fig. 3, lane 1). Next, we cloned mutated parts of the virus genomes and analyzed the DNA sequences. The results are shown in Fig. 4. They were as expected by the construction procedures of the plasmids. These three mutants (in205B, in205C, and dl205) have mutations on the coding sequence for the E1B-19K polypeptide and not on the coding sequence for the 54K polypeptide. These mutants could grow as efficiently as the WT virus in HEK (Table 1) or KB (data not shown) cells, unlike most of the host range mutants of Ad5.

Transcription and translation of mutants. To confirm the presence of the mutations, we examined the transcription and translation of the E1B mutant. mRNA in cells infected with mutants and WT virus at 14 h p.i. was analyzed with nuclease S1 mapping by using the 32P-labeled E1B probe (4.1 to 11.2 map units) (Fig. 5A). Since a great, excess amount of the DNA probe was used in RNA-DNA hybridization, the intensity of the bands was proportional to the amounts of the RNAs. Since the intensities of the bands were almost equal among cells infected with mutants and WT viruses, the levels of E1B mRNAs were estimated to be nearly equal among these cells. We next selected mRNAs complementary to E1A and E1B by hybridization with the HindIII-G of Ad12 DNA and translated the mRNAs eluted from the
hybrids by using a reticulocyte lysate system (Fig. 5). The E1a 38 to 40K polypeptides and E1B-19K and -54K polypeptides, identified in previous studies (21a), could be discerned, although the pictures contain some background present in mock-infected cells (Fig. 5B, panel e). The E1B-19K polypeptide synthesized by in205B was a little larger in size with a more basic pl value (Fig. 5B, panels a and b) than that synthesized by WT virus. Since the mutation in in205B is expected to result in the insertion of four amino acids (Pro-Asp-Pro-Asp), this result is rational. As for the other two mutants, in205C and dl205, we could not detect any polypeptide corresponding to the 19K polypeptide. Since the reading frames of the 19K polypeptide were shifted by insertion or deletion in these mutants, the 19K polypeptide was expected to be a 3K polypeptide, which was too small to be detected in the gel. Since the products of the E1A and 54K polypeptide encoded by E1B were detected in the same cells, the results may not be due to unfavorable conditions for translation in our system. The results are explained by the expected sequences in mutant DNA, and they show that the expected sequences are transcribed in cells infected with the mutants.

**Immunoprecipitation of the 19K polypeptide synthesized in cells infected with mutants.** KB cells were infected with mutants and WT virus labeled with [35S]methionine and harvested at 16 h p.i. From each of the cell extracts, gene products were immunoprecipitated with anti-19K sera (Fig. 6). We could detect the 19K polypeptide distinctly in the extract from the cells infected with WT virus (Fig. 6, panel a) (21a). However, we failed to detect polypeptides encoded by the E1B-19K polypeptide-coding sequences in extracts from all the cells infected with the mutants. In extracts from cells infected with in205C or dl205, the results can be explained by the fact that their sizes were too small to be detected (Fig. 6, panels c and d). In the extract from cells infected with in205B, the failure to detect the 19K polypeptide was unexpected (Fig. 6, panel b). There may be two possibilities to explain these results. One possibility is that the product is very unstable in KB cells, and the other is that the property of the product is changed so as not to be solubilized under the present condition. Further investigation will be required to solve the question.

**S1 mapping of mRNAs.** It is shown in the preceding section that all three mutants are not defective in replication. To confirm whether the above notion is correct, we carried out S1 mapping of viral mRNAs in permissive cells infected with the mutants (Fig. 7). The results indicate that mRNA patterns at early and late stages in cells infected with the mutants were the same as those in cells infected with WT virus. Thus, transcriptional control is not affected by the mutation.

**FIG. 3.** Restriction endonuclease cleavage patterns of the viral DNAs from mutants. Viral DNAs from WT virus (lanes a, e, and i), in205B (lanes b, f, and j), in205C (lanes c, g, and k), and dl205 (lanes d, h, and l) were cleaved with restriction endonucleases and analyzed by electrophoresis in a 1% agarose gel with Tris-acetate-EDTA buffer. The bands were visualized by staining with ethidium bromide. When DNAs were cleaved with BamHI (lanes a to d), WT virus (lane a), and in205C (lane c) DNAs showed similar patterns. Two extra bands were detected in in205B DNA (the 6,420- and 1,600-bp bands; lane b) and dl205 DNA (the band which is a little shorter than 6,420 bp and the 1,600-bp band; lane d) with the disappearance of the BamHI-A band (8,020 bp) in WT DNA (lane a), indicating the introduction of new BamHI sites in in205B and dl205 DNAs. When DNAs were cleaved with Clal (lanes e to h), the new band (1,600 bp) was detected only in in205C DNA (lane g), whereas in other DNAs (lane e for WT DNA, lane f for in205B DNA, and lane h for dl205 DNA) only the bands of the full genome length (34,000 bp) were detected, indicating the presence of the new Clal site in in205C DNA and the absence of the site in WT, in205B, and dl205 DNAs. When DNAs were cleaved with HindIII (lanes i to l), the cleavage patterns of in205B DNA (lane j) and in205C DNA (lane k) were the same as that of WT DNA (lane i). In dl205 DNA (lane l), the HindIII-G band (2,320 bp in WT DNA) is a little short to be 2,140 bp because of the deletion of 175 bp.

**FIG. 4.** DNA and the corresponding amino acid sequences of the mutated viruses. The mutated parts of the virus genomes were cloned in pBR322, and the nucleotide sequences were analyzed. Underlines show the DNA sequences derived from linker sequences. The numbers indicate bp positions counted from the left end of the virus genome. Symbols: ***, cutting sites of restriction enzymes; ***, termination codons; -- -- --, the DNA or amino acid sequence continues.
TABLE 1. Growth of WT virus and mutants in HEK cellsa

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus yield (PFU/ml)</th>
<th>Expt I</th>
<th>Expt II</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1.0 × 10⁸</td>
<td>1.7 × 10⁷</td>
<td></td>
</tr>
<tr>
<td>in205B</td>
<td>2.6 × 10⁸</td>
<td>6.5 × 10⁷</td>
<td></td>
</tr>
<tr>
<td>in205C</td>
<td>2.6 × 10⁸</td>
<td>4.5 × 10⁷</td>
<td></td>
</tr>
<tr>
<td>d1205</td>
<td>2.2 × 10⁸</td>
<td>8.0 × 10⁷</td>
<td></td>
</tr>
</tbody>
</table>

a HEK cells were infected at ca. 5 to 10 PFU per cell, and virus yields in cell-medium lysates at 72 h p.i. were measured by plaque assay in HEK (experiment I) or 293 (experiment II) cells.

Transformation of cells with mutants. The abilities of the mutants to transform rat 3Y1 cells were examined. As shown in Fig. 8, the mutants could induce much reduced numbers of foci compared with those induced by WT virus.

We then subcultured the transformed cells from the foci and established transformed cell clones. The ability of cells in each clone to grow in soft agar medium was tested. As shown in Table 2, most of the transformed cell clones induced by the mutants failed to form colonies in soft agar medium, whereas most of those induced by WT virus could grow to form colonies of distinct sizes in soft agar medium. These results indicate that both the frequency of cell transformation and the phenotype of transformed cells were different between the mutants and the WT virus.

Next, tumor formation in vivo was assayed. The virus suspensions were injected subcutaneously into baby hamsters. As shown in Table 3, the tumorigenesis of the mutants was much reduced compared with that of the WT virus. The results indicate that the three mutants have much reduced abilities to transform rodent cells both in vitro and in vivo.

DISCUSSION

We isolated three Ad12 mutants with either insertions or a deletion in the E1B-19K polypeptide-coding sequence. For isolation, the DNA-protein complex was cleaved at a single site and ligated with DNA fragments mutated on the sequence coding for the E1B-19K polypeptide. HEK cells were transfected to isolate mutants. At first, we expected that the mutant would be obtained as a mixed virus with a WT, because it was known that most Ad5 mutants defective in E1B grow in 293 cells (8) but not in HEK or HeLa cells (11, 13). Unexpectedly, we found that Ad12 mutants defective in the E1B-19K polypeptide could grow efficiently in HEK cells. This finding enabled us to isolate mutants with the use of only HEK cells and without the use of 293 cells. Mutants were selected by restriction endonuclease cleavage patterns of their DNAs and established after plaque purification in HEK cells. The reason for the difference between Ad5 and Ad12 E1B mutants is not known. However, careful comparison of mutants suggests that the larger polypeptide (54K) encoded by E1B is required and that the smaller polypeptide (15K or 19K) encoded by E1B is not required for virus growth. Ad5 dl13 is defective in virus growth and in the synthesis of the larger polypeptide (13), whereas Ad12 E1B mutants discussed above are nondefective in virus.

FIG. 5. Transcription of the E1 gene in cells infected with the mutants. (A) Sl gel analysis of E1B mRNAs. The cytoplasmic RNAs were extracted from KB cells infected with WT virus and mutants at 14 h p.i. and hybridized with 32P-labeled E1B probe (4.1 to 11.2 map units). The Sl-resistant DNA-RNA hybrids were analyzed by electrophoresis in a 1.4% agarose gel with alkaline buffer. The bands of 1,760 nucleotides are E1B mRNAs in cells infected with WT virus (lane a), in205B (lane b), and in205C (lane c). The band a little shorter than 1,760 nucleotides is E1B mRNA in cells infected with dl205 (lane d). The lower bands are mRNAs for polypeptide IX. (B) In vitro translation of mRNAs complementary to E1. The mRNAs complementary to E1 were selected by hybridization with HindIII-G of Ad12, eluted and translated in vitro. The products were analyzed by two-dimensional gel electrophoresis. The polypeptides were separated by pH gradients from the left (pH 7.5) to the right (pH 4.0) and by molecular weight from top to bottom. Arrows indicate the products of E1: 38 to 40K, the polypeptide encoded by E1A (T antigen g); 19K, 19.5K, and 54K, the polypeptides encoded by E1B. (a) WT; (b) in205B; (c) in205C; (d) dl205; (e) mock infected.
growth and in the synthesis of the larger polypeptide. To substantiate this suggestion, the isolation of Ad12 E1B mutants defective in the synthesis of 54K polypeptides is necessary.

The first mutant, in205B, has an insertion of 12 bp (4 amino acids, in frame) at the Accl site (4.5 map units). The second mutant has an insertion of 16 bp (frame shift) at the same site. The third mutant, d1205, has a deletion of 175 bp (sequence between 4.5 and 5.1 map units; frame shift). From sequence data, it is expected that in205B will produce a longer (4 amino acids) polypeptide and that in205C and d1205 will produce polypeptides that are as small as 3K, instead of the E1B-coded 19K polypeptide detected in WT-infected cells (Fig. 4).

After infection of KB cells with mutants, transcription of the E1B gene was found to proceed efficiently in the same way as in WT infection. In vitro translation of E1B mRNA revealed that in205B produces a polypeptide that is a little longer and more basic than the 19K polypeptide and that in205C and d1205 produce polypeptides that are too small to be detected under the present condition. This observation agrees with the expectation. When the extract of cells infected with in205B and labeled with [35S]methionine was immunoprecipitated and analyzed in gel electrophoresis, no specific band or spot was detected. This observation may be explained by the alteration in stability or solubility or both of the gene product.

Although all three mutants replicate in permissive cells as efficiently as in WT cells, the mutants show much reduced capacities for cell transformation. This fact was confirmed by the very low frequencies in the appearance of transformed cell foci in rat cells, no or poor formation of colonies in soft agar culture with cells transformed by the mutants,
TABLE 2. Colony formation by transformed cells in soft agar culture∗

<table>
<thead>
<tr>
<th>Cells transformed by:</th>
<th>Duration (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>WT</td>
<td>3/5</td>
</tr>
<tr>
<td>in205B</td>
<td>0/4</td>
</tr>
<tr>
<td>in205C</td>
<td>0/3</td>
</tr>
<tr>
<td>d205</td>
<td>0/6</td>
</tr>
</tbody>
</table>

∗Cells transformed by WT virus and mutants were subcultured from each of the foci and tested for colony formation in soft agar cultures for 2 and 3 weeks. The denominator is the number of transformed cell clones tested, and the numerator is the number of transformed cells. Each colony is involved in at least 104 PFU virus and the corresponding virus culture contains at least 104 PFU virus. The virus titers shown was injected subcutaneously into baby hamsters and observed for tumor induction for 90 days. The denominator is the number of hamsters observed, and the numerator is the number of hamsters with tumors. ND. Not determined.

TABLE 3. Tumor induction in hamsters by Ad12 WT virus mutants∗

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dose (PFU)</th>
<th>10³</th>
<th>10⁴</th>
<th>10⁵</th>
<th>10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0/2</td>
<td>2/11</td>
<td>5/6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>in205B</td>
<td>ND</td>
<td>ND</td>
<td>0/11</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>in205C</td>
<td>ND</td>
<td>ND</td>
<td>0/11</td>
<td>0/12</td>
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</tr>
<tr>
<td>d205</td>
<td>ND</td>
<td>0/12</td>
<td>0/6</td>
<td>0/10</td>
<td></td>
</tr>
</tbody>
</table>

∗The virus with titers shown was injected subcutaneously into baby hamsters and observed for tumor induction for 90 days. The denominator is the number of hamsters observed, and the numerator is the number of hamsters with tumors. ND. Not determined.

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