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Genetic and Structural Analysis of a Virulence Determinant in Polyomavirus VP1

PAUL H. BAUER,[1] ROD T. BRONSON,[2] SHUI C. FUNG,[1] ROBERT FREUND,[3] THILO STEHLE,[4] STEPHEN C. HARRISON,[4] AND THOMAS L. BENJAMIN[1]*

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115[1]; Department of Pathology, Tufts University School of Veterinary Medicine, Boston, Massachusetts 02111[2]; Department of Microbiology and Immunology, University of Maryland at Baltimore School of Medicine, Baltimore, Maryland 21201[3]; and Howard Hughes Medical Institute and Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138[4]

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The LID strain of polyomavirus differs from other laboratory strains in causing a rapidly lethal infection of newborn C3H/Bi mice. This virulent behavior of LID was attenuated by dilution, yet at sublethal doses LID was able to induce tumors at a high frequency, like its parent virus PTA. By constructing and assaying LID-PTA recombinant viruses and by DNA sequencing, the determinant of virulence in LID was mapped to the major viral capsid protein, VP1. The VP1s of LID and PTA differed at two positions: at 185, LID has phenylalanine and PTA has tyrosine, and at 296, LID has alanine and PTA has valine. Results obtained with viruses constructed by site-directed mutagenesis showed that alanine at position 296 is sufficient to confer a fully virulent phenotype regardless of which amino acid is at position 185. However, with valine at position 296, an effect of phenylalanine at position 185 is apparent, as this virus possesses an intermediate level of virulence. A crystal structure of polyomavirus complexed with 3′-sialyl lactose previously indicated van der Waals contacts between the side chain of valine 296 and the sialic acid ring (T. Stehle, Y. Yan, T. L. Benjamin, and S. C. Harrison, Nature [London] 369:160–163, 1994). When this interaction was modeled with alanine, these contacts were greatly reduced. Direct confirmation that the substitutions in VP1 affected receptor binding was obtained by studying virus hemagglutination behavior. The ensemble of results are discussed in terms of the idea that a lower affinity of the virus for its receptor can result in more rapid spread and increased pathogenicity.

Efforts in several laboratories have identified various determinants in mouse polyomavirus that affect its pathogenicity. Among wild-type laboratory strains that replicate and transform well in culture, some are highly tumorigenic in mice whereas others are only weakly so (7). A determinant responsible for efficient virus spread and induction of tumors has been mapped to a single amino acid in the major capsid protein VP1 (9, 13). Determinants that alter the spectrum of tissues in which polyomavirus can replicate in the mouse have also been mapped to VP1 (22). Mutations in the tumor antigens that block or reduce the virus's ability to transform cells in vitro (18, 23) inhibit or alter its tumorigenicity in the mouse (9, 11, 14, 30). Determinants that affect viral pathogenicity have also been found in the noncoding region of the virus. These determinants influence the ability of the virus to induce tumors of the thymus (10, 13) or to replicate and persist in the kidney (1, 25, 26).

A laboratory isolate of polyomavirus known as LID is unique in that it causes a rapidly lethal infection of newborn mice. Originally derived from the high-tumor strain PTA by repeated passage in cultures of mouse embryo cells and in newborn mice (20, 27), LID causes death within 1 to 2 weeks primarily from kidney failure and brain hemorrhages (2). The Kilham mouse virus is also lethal when injected into newborn mice, but it produces death due to interstitial pneumonia (16), and its genome is more similar to that of simian virus 40 than to that of the mouse polyomavirus of interest here (21).

The molecular basis of the virulent behavior of LID is not understood. Identification of the determinant(s) of virulence could provide important information about the interaction of polyomavirus with its natural host. The goals of this study were to characterize the pathology of mice infected with a molecularly cloned stock of LID, to map the virulence determinant(s), and to interpret as far as possible the mechanism of virulence.

MATERIALS AND METHODS

Mice and virus injections. All mice were of the inbred strain C3H/BiDa, obtained from a polyomavirus-free breeding colony. Newborn mice (less than 18 h of age) were injected intraperitoneally with 50 μl of viral stock per animal. Only those mice that survived at least 3 days postinfection (dpi) were included in the results. For studies of virulence, survival was checked daily for a period of up to 50 days. At necropsy, one kidney was kept at −20°C for confirmation of the viral genotype. For studies of tumorigenicity, the mice were allowed to live until they became moribund as a result of development of tumor(s). Mice were necropsied within 18 h of death or immediately after being sacrificed. All tumors were confirmed histologically.

Cloning of viruses and production of stocks. LID was provided by Clyde Dawe; PTA has been described previously (7). Methods used for production of cloned viral stocks have been described elsewhere (7, 13) and are briefly summarized below. Viral DNAs were isolated from crude viral stocks and subcloned into bacterial vectors; single clones were used for production of viral stocks. Cloned viral DNAs were transfected into NIH 3T3 cells to produce virus, which was further cloned by plaque isolation on NIH 3T3 cells. Viral stocks were made on baby mouse kidney cells infected with virus from a single plaque. Stocks were titered at least twice by plaque assay. Recombinant viral stocks were produced by ligation of agarose gel-purified LID and PTA genomic fragments as indicated, followed by transfection and isolation of plaques. Viral stocks containing site-directed mutations in VP1 were produced similarly, with mutagenesis performed

* Corresponding author. Mailing address: Department of Pathology, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: (617) 432-1959. Fax: (617) 277-5291. Electronic mail address: bauer@mbcrr.harvard.edu.

in bacteria on the *Eco*RI-*Bam*HI fragment of the viral genome containing the VP1 gene, using a Transformer kit (Clontech).

Confirmation of viral genotype. The genotype of virus present in stocks prior to injection as well as in kidneys of infected mice was confirmed to screen for accidental contamination or reversion. The identities of amino acids 185 and 296 of VP1 were determined by sequencing DNA corresponding to these regions after PCR amplification. The starting material for PCR was either 2 μl of crude viral stock or 2 μl of kidney homogenate. The material was mixed with 20 μl of GeneReleaser (BioVentures), microwaved for 6 min, and held at 80°C before the appropriate primers and reagents for PCR were added. PCR was performed with a GeneAmp kit (Perkin-Elmer) for 40 cycles under standard conditions, and DNA fragments were isolated by electrophoresis on a 2% NuSieve (FMC BioProducts) agarose gel (5). DNA sequencing was performed with a Sequenase kit (United States Biochemical) directly on the DNA in the gel slice (31).

Whole mouse section hybridization and histopathology. Whole mouse section hybridization and autoradiography were performed on sections cut from mice embedded in the Tissue-Tek OCT compound (Miles) as previously described (8). Sections were hybridized with polyomavirus DNA labeled with [^{35}S]dCTP by random priming (Boehringer Mannheim). Histopathology was performed on tissues that were fixed in Bouin's solution and paraffin embedded as previously described (7). Sections were either stained with Gill's hematoxylin and eosin or left unstained for immunohistochemistry. Immunohistochemistry was performed with the peroxidase-antiperoxidase procedure (Dako PAP kit) with a polyclonal rabbit antiserum against polyomavirus VP1 as the primary antibody.

HA assays. Hemagglutination (HA) assays at five pHs were performed with virus stocks diluted in phosphate-buffered saline, using 0.4% guinea pig erythrocytes as previously described (12). Plates were read after overnight incubation at 4°C.

Genomic sequencing. Sequencing of the genomes of LID and PTA was performed at the Biopolymer Laboratory at the University of Maryland at Baltimore School of Medicine. Polyomavirus genomic DNA subcloned into bacterial vectors was sequenced with an Applied Biosystems automatic sequencer in both directions, using staggered primers approximately 150 nucleotides apart.

Nucleotide sequence accession numbers. The GenBank accession numbers for the PTA and LID genomes are U27812 and U27813, respectively.

RESULTS

Virulent and tumorigenic behavior of molecularly cloned LID. The LID strain of polyomavirus has been shown to cause acute morbidity in a dose-dependent fashion when injected into newborn mice (2). To map the determinant of LID responsible for this effect, viral genomic DNA was first cloned and used to generate virus. Viral stocks were obtained from three individual plaques, and each of these was injected into a single litter of mice. The acute morbidity induced by the uncloned stock of LID was replicated with all three cloned stocks (data not shown), and one of these was chosen for all further experiments.

The dependence of acute morbidity on the dose of LID was investigated by inoculating the virus at various dilutions. A cloned stock of PTA, the parent virus of LID, was prepared in a similar manner and injected at a single dose as a control. Whereas none of the mice injected with undiluted PTA died within the 50-day period of observation, all mice injected with an equivalent dose of LID died by 8 dpi (Fig. 1). In repeated experiments, mice injected with undiluted LID had a mean survival time of 9 ± 3 days (n = 38). All mice injected with a 1:3 or 1:10 dilution of LID died between 8 and 15 dpi. At higher dilutions of 1:30 and 1:100, deaths occurred later, and some animals survived to at least 50 dpi. The dose-dependent virulence of LID stands in contrast to the dose-independent tumorigenicity of PTA, which produces a similar tumor profile over a dose range of approximately 10^1 to 10^6 PFU per animal (reference 7 and unpublished results).

To determine if LID retains the tumor-inducing potential of PTA, a sublethal dose of 2×10^3 PFU per animal was inoculated into newborn mice. This dose was chosen since it is low enough to avoid early lethality but well within the range at which PTA induces a full tumor profile. Over 95% of these mice developed tumors, which were of both epithelial and mesenchymal origin (Table 1). Compared with PTA at a similar dose (7), LID induced tumors at all of the same sites except

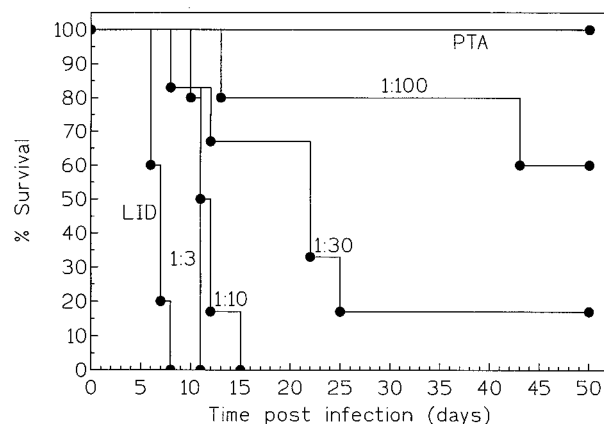

FIG. 1. Survival of mice infected as newborns with PTA and LID. LID (3×10^7 PFU/ml) was diluted as indicated in Dulbecco's modified Eagle's medium containing 2% calf serum. Fifty microliters of each dilution was injected into each of four to six mice. Fifty microliters of undiluted PTA (2×10^7 PFU/ml) was injected into four mice.

the thymus, where no tumors were seen. LID induced salivary gland tumors at a lower frequency and kidney tumors at a higher frequency than PTA. These differences may not be significant, however, given the relatively small number of animals used and the normal variations in tumor frequencies seen with different stocks of the same virus.

Levels of virus replication and spread were compared between LID and PTA by in situ hybridization of whole mouse sections and by immunoperoxidase staining for VP1 in various tissues. Both viruses have established disseminated infections by 12 dpi, but LID has replicated to higher levels than PTA throughout the mouse and caused significant runting (Fig. 2). The severities of infection by LID and PTA were compared at various sites 10 to 15 dpi by determining the degree of lytic damage and by immunostaining for VP1. Lytic lesions caused by LID were clearly more extensive and severe than those caused by PTA at each of the sites examined (Table 2). Similar findings with LID in the brain and kidney at 8 dpi have been reported previously (2). In the kidney, the major site of virus amplification, not only is the degree of infection with LID greater, but the pattern of infection within the organ differs as well. At 10 to 15 dpi, PTA infects primarily the corticomedullary junction of the kidney, whereas LID infects both the medulla and cortex to a much greater extent (Fig. 3A and B; also see Fig. 2). At later times (25 dpi), PTA is able to infect the cortex of the kidney, but not to the degree seen with LID (data not shown). As noted previously, destruction of kidney tissue appears to be a major factor leading to death in newborn mice inoculated with LID (2). Severe thyroid lesions may contribute to mortality as well.

LID also induces lytic lesions in endothelial cells of capillaries in the brain, a site rarely infected by PTA. These lesions are the probable cause of the brain hemorrhages characteristic of LID disease (Fig. 3C). This hypothesis is supported by the presence of VP1 in brain endothelial cells (Fig. 3D). In many cases, the hemorrhages are small and probably not severe enough to cause death.

The determinant of virulence maps to VP1. As a step toward identifying the determinant(s) of virulence, the entire genomes of LID and PTA were sequenced. The genome of LID was 5,308 bp in length, compared with 5,380 bp for PTA. Most of the differences between the two genomes were located in noncoding sequences flanking the origin of replication (Fig. 4A).

TABLE 1. Tumor induction in mice infected by LID or PTA[a]

Virus	Mice analyzed			Distribution (%)						
				Epithelial tumor				Mesenchymal tumor		
	No. injected	Mean age at necropsy (days)	% with tumor(s)	Hair follicle	Thymus	Mammary gland	Salivary gland	Fibrosarcoma	Renal medulla	Bone
LID	23	114	96	22	0	22	4	17	48	43
PTA	13	190	100	54	31	8	38	15	8	31

[a] Newborn mice were infected with 2×10^3 to 5×10^3 PFU per animal. The data for PTA have been previously published (7).

Noncoding sequences on the early side of the replication origin were identical except that LID had only one copy of a 40-bp sequence that is duplicated in PTA. The lack of this duplication reduces the number of predicted binding sites for polyomavirus large T antigen from four in PTA to three in LID (28). This duplication was previously shown to be important to the ability of PTA to induce thymic tumors (10), and its absence from LID explains the inability of diluted LID to induce this particular tumor type (Table 1).

The A enhancer on the late side of the replication origin is identical between LID and PTA, while the B enhancer is substantially different. The B enhancer of LID contains three unique insertions not found in PTA, while PTA contains five unique insertions of its own. In addition, there are 15 single-nucleotide substitutions between the two genomes spread throughout this region. Duplications and mutations in this enhancer are common among different strains of polyomavirus (28), and some of these have been shown to affect organ-specific viral DNA replication and persistence (1, 25, 26). The combination of the B-enhancer insertions and the lack of the 40-bp duplication on the early side of the replication origin account for the difference in length of the two genomes.

In contrast to the origin region, the coding regions of the two viral genomes show few differences. Eight single-nucleotide substitutions were found spread over 4,818 bp: one in VP2, four in VP1, and three in large T. Of these eight, only two in VP1 result in a change of amino acid: an A→G transition that changes Val at position 296 in PTA to Ala in LID, and a T→A transversion that changes Tyr at position 185 in PTA to Phe in LID.

To localize the region in LID responsible for its virulence, recombinant viruses were constructed and tested in mice. The *Kpn*I-*Hin*dIII fragment of LID encoding the C-terminal portions of VP1 and large T antigen contained the virulence determinant, since a virus containing this fragment of LID in a PTA background (PTA-LKH) caused mice to die with a time course similar to that of wild-type LID (Fig. 4B). Conversely, early lethality was not seen in a reciprocal recombinant containing the same fragment of PTA in a LID background (LID-PHK). These results rule out origin differences and point to one or both of the VP1 coding differences as the likely determinant of virulence.

Site-directed mutagenesis was used to confirm the importance of the VP1 changes and to assess their individual contributions to the virulence of LID (Fig. 5). The viruses can be divided into three groups. The first group includes only PTA and is nonvirulent, rarely causing lethality before 50 dpi. The second group includes LID-A296V and PTA-Y185F. These two viruses, with identical VP1 amino acid sequences that include Phe-185 and Val-296, show a degree of virulence intermediate between those of PTA and LID. The third group of viruses includes LID, LID-F185Y, and PTA-V296A. These viruses all show the fully virulent phenotype, and all encode Ala-296. Ala-296 is therefore the primary determinant of virulence of LID. The data also show that Phe-185 is a secondary or conditional determinant, able to increase virulence when the primary determinant is absent, i.e., when linked to Val-296.

A histopathological examination at 10 to 15 dpi indicated that mice infected with viruses containing Ala-296 (LID-F185Y and PTA-V296A) show the same pattern and severity of lytic lesions as wild-type LID (Table 2 and Fig. 3). This finding supports the conclusion that amino acid 296 is the primary determinant of virulence. The pathology of animals injected with the two viruses of intermediate virulence containing Phe-185 and Val-296 (LID-A296V and PTA-Y185F) was dependent on the time postinfection at which the mice died. Mice that died before 15 dpi showed the same pattern and severity of lytic lesions as wild-type LID, including the presence of brain hemorrhages. Mice that survived to 24 dpi showed a reduced severity of lesions compared with mice that

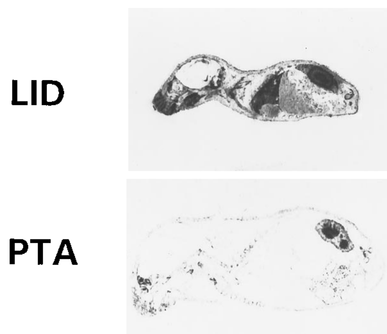

FIG. 2. Whole mouse sections of infected mice. Newborn mice infected with undiluted LID or PTA were sacrificed at 12 dpi. Hybridization of sections was performed with ^{35}S-labeled polyomavirus DNA, and autoradiography was performed for 16 h.

TABLE 2. Extent and severity of lytic lesions in mice infected by LID or PTA[a]

Site	Extent or severity of lytic lesions	
	LID	PTA
Salivary gland	Mild	Rare
Brain endothelium	Mild	Rare
Cartilage	Moderate	Rare
Skin	Moderate	Mild
Tooth bud	Severe	Rare
Heart	Severe	Mild
Bone	Severe	Mild
Vascular smooth muscle	Severe	Mild
Thymus	Severe	Mild
Lung	Severe	Mild
Thyroid	Severe	Mild
Kidney	Severe	Moderate

[a] Newborn mice were infected with 1×10^6 to 2×10^6 PFU per animal and sacrificed at 10 to 15 dpi. Lytic lesions were graded on a four-point scale based on the number of VP1-positive cells: rare to mild to moderate to severe.

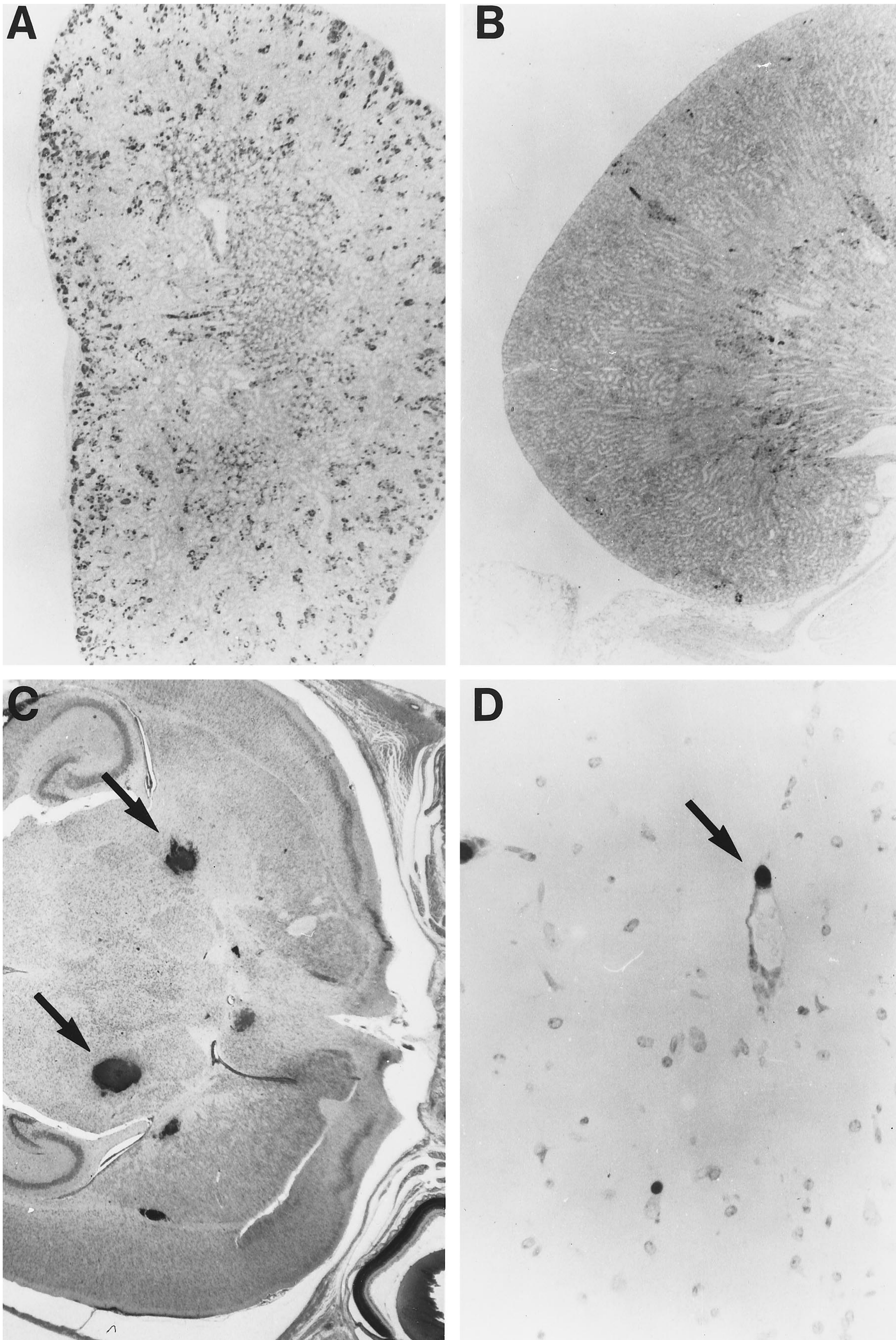

FIG. 3. Pathology in kidney and brain of infected mice. Immunohistochemistry for VP1 was performed on kidney sections from mice infected with undiluted LID (A) or PTA (B) at 12 dpi (magnification, ×10). (C) A tissue section of brain from a LID-infected mouse at 12 dpi was stained with hematoxylin and eosin (magnification, ×5). Hemorrhages typical of LID infection are marked with arrows. (D) Immunohistochemistry for VP1 was performed on a brain section from a LID-infected mouse at 12 dpi (magnification, ×400). The presence of VP1 in an endothelial cell is indicated by an arrow.

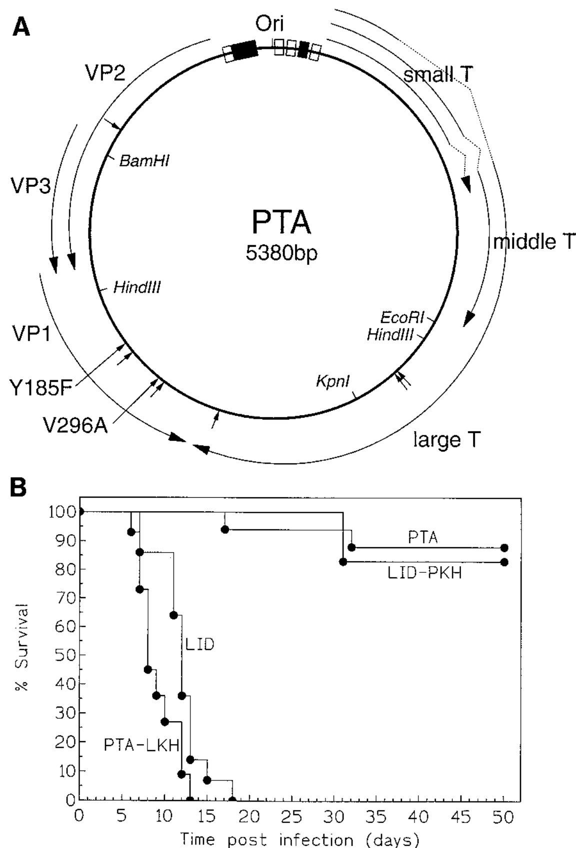

FIG. 4. Schematic showing differences between the PTA and LID genomes (A) and survival of newborn mice infected with parental and recombinant viruses (B). (A) Nucleotide differences between LID and PTA in the coding region are indicated by small arrows on the map. The boxes on the early side of the replication origin are predicted large-T-antigen binding sites. The filled box indicates the site missing in LID. The boxes on the late side of the replication origin indicate enhancers A (open box) and B (filled box). See text. (B) Viruses were injected into each of 6 to 16 mice at 1×10^6 to 2×10^6 PFU per animal. In the LID-PKH recombinant virus, the *Kpn*I-*Hin*dIII fragment of LID encoding the majority of VP1 has been replaced by the corresponding fragment of PTA. The PTA-LKH virus is the reciprocal recombinant.

died earlier, although the lesions were still more severe than those found in PTA-infected mice of the same age. These mice have small brain hemorrhages and also show preneoplastic lesions in tissues that are sites of tumor development in mice injected with diluted LID (Table 1).

Predictions based on the structure of VP1. An examination of the X-ray crystal structure of polyomavirus complexed with 3′-sialyl lactose indicates that amino acid 296 of VP1 is directly involved in binding the sialic acid moiety (29). A specific protein receptor on host cells for polyomavirus has not been identified, but the presence of sialyloligosaccharides on the cell surface is required for productive infection and for hemagglutination (3, 15). The oligosaccharide lies in a narrow groove on the virus surface. There are several contacts between polar amino acid side chains of VP1 and the *N*-acetyl and carboxylate substituents of the sialic acid, and there is an overall van der Waals complementarity of the oligosaccharide and the groove. One face of the sialic acid lies against a nonpolar surface of VP1 that contains residue 296. The side chain of Val-296 is in van der Waals contact with C-3 and O-4 of the sialic acid. When this interaction is modeled with Ala-296, these contacts essentially disappear (Fig. 6). The replacement of Val-296 by Ala removes both methyl groups and places the common β-carbon more than 4 Å (0.4 nm) away from the nearest sialic acid atom, O-4. This substitution therefore significantly reduces the hydrophobic interaction between this residue and the sialic acid.

Tyr-185 is located in a region buried beneath the oligosaccharide binding groove at the interface between VP1 monomers (29). This residue is solvent inaccessible, and from its position in the structure, any direct contact between it and a sugar can be ruled out. The hydroxyl group of Tyr-185 is located in a highly polar environment and is involved in a hydrogen bond network with several charged residues. Substitution of Phe at this position would disrupt this network and could potentially affect the conformation of the overlying receptor binding region.

Effects of VP1 mutations on receptor binding. To demonstrate directly that the differences in VP1 between LID and PTA affect the ability of the virus to interact with receptors on target cells, the abilities of the wild-type and mutant viruses to agglutinate guinea pig erythrocytes at different pHs were examined (Fig. 7). HA by polyomavirus shows the same specificity for sialyloligosaccharides as infection of host cells (3, 15). The HA data show that the viruses fall into the same categories as determined by assays of virulence (Fig. 5). The first group, consisting of PTA alone, shows an increase in HA titer with increasing pH; the second group, containing LID-A296V and PTA-Y185F, shows an increase and then a decrease in HA titer with increasing pH, and the third group, containing LID, LID-F185Y, and PTA-V296A, shows a decrease in HA titer with increasing pH. Consistent correlations therefore exist between the identity of the amino acids at 185 and 296, the HA profiles, and the degree of virulence of the viruses in mice. These results clearly point to an alteration in virus-receptor interaction as the basis for the virulent behavior of LID and are consistent with the interpretation of the virus-receptor cocrystal structure.

DISCUSSION

The LID strain of polyomavirus was derived from the highly tumorigenic PTA strain but differs from the latter in causing a rapidly lethal infection of newborn mice (2). Here, LID has been rederived by molecular cloning and compared with a

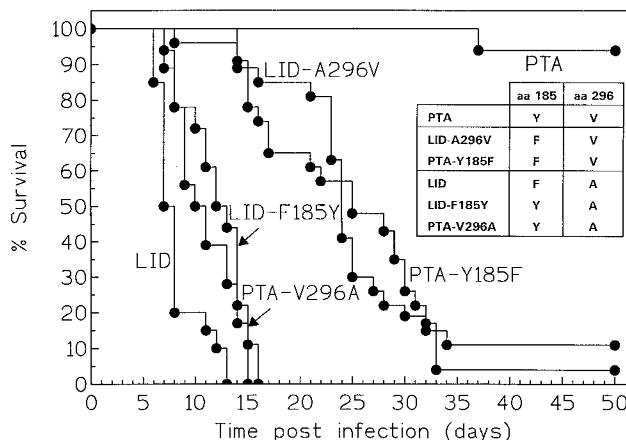

FIG. 5. Survival of mice infected as newborns with parental and site-directed mutant viruses. Viruses were injected into each of 17 to 27 mice at 1×10^6 to 2×10^6 PFU per animal. The inset shows the identities of amino acids (aa) 185 and 296 of VP1 of the six viruses injected.

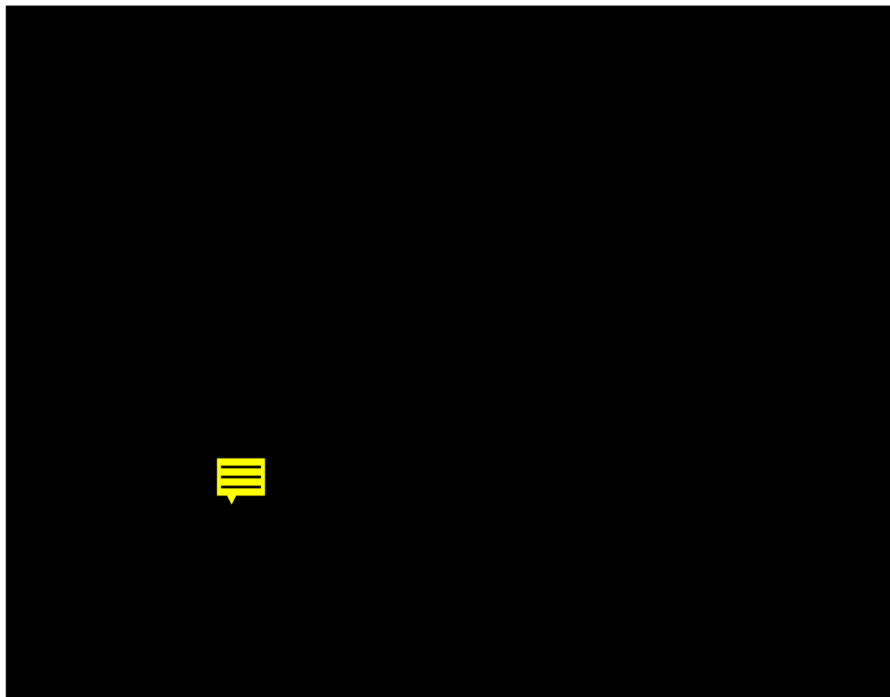

FIG. 6. The receptor binding site of polyomavirus VP1 complexed to a sialyloligosaccharide. The filled balls are the sialic acid moiety of the receptor fragment, with carbon atoms yellow and oxygen atoms red. Both Val (dotted red surface) and Ala (dotted green surface) at position 296 of VP1 are shown. The figure has been generated with the computer program O (17), and the dotted-surface representations have been generated by the computer program MS (6), using a radius of 2.1 Å (0.21 nm) for carbon atoms. The view is such that the galactose moiety of the receptor fragment would reside above the plane of the paper.

similarly cloned PTA in order to identify the basis of its virulence. Newborn mice inoculated with LID at doses of $\geq 10^5$ PFU per animal succumbed within 2 weeks. However, at lower doses, animals survived and went on to develop an array of tumors similar to those induced by PTA. The LID strain has thus acquired virulent properties without losing the ability to induce tumors. Whole mouse section hybridization and immunohistochemical staining for viral capsid protein VP1 showed that LID disseminated more rapidly and caused more tissue damage than PTA at each of a dozen sites examined.

Sequencing of the LID and PTA genomes revealed that the two viruses differed extensively in their regulatory sequences around the origin of replication, particularly in the B enhancer, while the coding regions contained only eight substitutions, six of them silent. By constructing and testing LID-PTA recombinant viruses, the determinant of virulence was shown to be in VP1. This result rules out the differences in the regulatory region around the origin as having any significant effect on virulence, although a role for these differences in determining frequencies of individual tumor types cannot be ruled out.

The VP1s of LID and PTA differ by two conservative amino acid substitutions: at position 185, LID encodes Phe and PTA encodes Tyr, and at position 296, LID encodes Ala and PTA encodes Val. Site-directed mutagenesis was used to confirm the importance of the VP1 changes in causing virulence and to determine the contributions made by each of the substitutions. The results showed that the presence of Ala at 296 in LID was the primary determinant, since introducing this change alone on a PTA background resulted in full virulence. A secondary effect of Phe-185 could be seen, but this was conditional upon

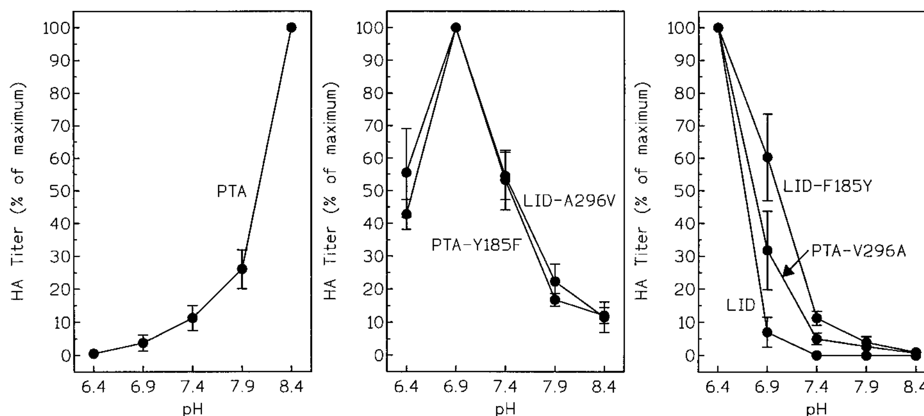

FIG. 7. HA behavior of LID, PTA, and site-directed mutant viruses. Each point is the mean ± standard of the mean of four independent experiments.

Val being present at 296. Thus, converting Tyr-185 to Phe on a PTA background or Ala-296 to Val on an LID background resulted in an intermediate level of virulence.

The changes in VP1 clearly affect cell binding, as shown by studies of the HA properties of viruses bearing one or both of the LID substitutions. LID and highly virulent recombinants with Ala-296, viruses of intermediate virulence with Val-296 and Phe-185, and nonvirulent PTA all gave distinctive HA profiles with changes in pH on guinea pig erythrocytes. While the different HA profiles cannot be rationalized directly in terms of the particular amino acid substitutions, the latter must affect virus-cell binding through some pH-dependent rearrangement in the receptor binding portion of VP1.

The attachment of polyomavirus to cells requires binding of VP1 to terminal sialic acid residues in an α-2,3 linkage on the cell surface (15). LID should bind sialic acid with reduced avidity compared with PTA, since the crystal structure of polyomavirus bound to 3′-sialyl lactose predicts that the Ala-for-Val substitution at 296 removes important hydrophobic interactions with the sialic acid ring. How the apparently weaker binding of LID to receptors is translated into more rapid and extensive virus spread and a virulent phenotype is not clear. Too high an avidity of the virus for its receptor could inhibit virus entry through an effect on some step along the pathway of internalization, delivery to the nucleus, and uncoating. Too high an avidity for receptor could also inhibit virus escape from the infected cell, resulting in less efficient and less rapid spread. In this regard, influenza virus, which also requires cell surface sialic acid for attachment, relies on its neuraminidase as a receptor-destroying enzyme to aid in virus release and facilitate its spread to other cells (19, 24). Small changes in receptor avidity, particularly in a virus such as polyomavirus that lacks a receptor-destroying enzyme, could have a significant effect on the efficiency of virus release and spread and thus on virulence.

Polymorphisms in VP1 underlie an enormous range of pathogenicity of polyomavirus. In an earlier study in which the highly tumorigenic strain PTA was compared with the weakly tumorigenic strain RA, the important determinant underlying efficient virus spread and tumor induction was mapped to VP1. As in the case of LID and PTA, a single amino acid substitution was responsible for the biological differences between PTA and RA (9, 12, 13). While the PTA-RA substitution does not alter contacts between VP1 and the terminal sialic acid, it does affect the ability of the virus to discriminate between branched and unbranched sialyloligosaccharides (4, 29). Additional studies directed at identification and characterization of the polyomavirus receptor(s) as well as the processes of virus entry and release are needed to understand more fully how these polymorphisms in VP1 lead to disease in the host.

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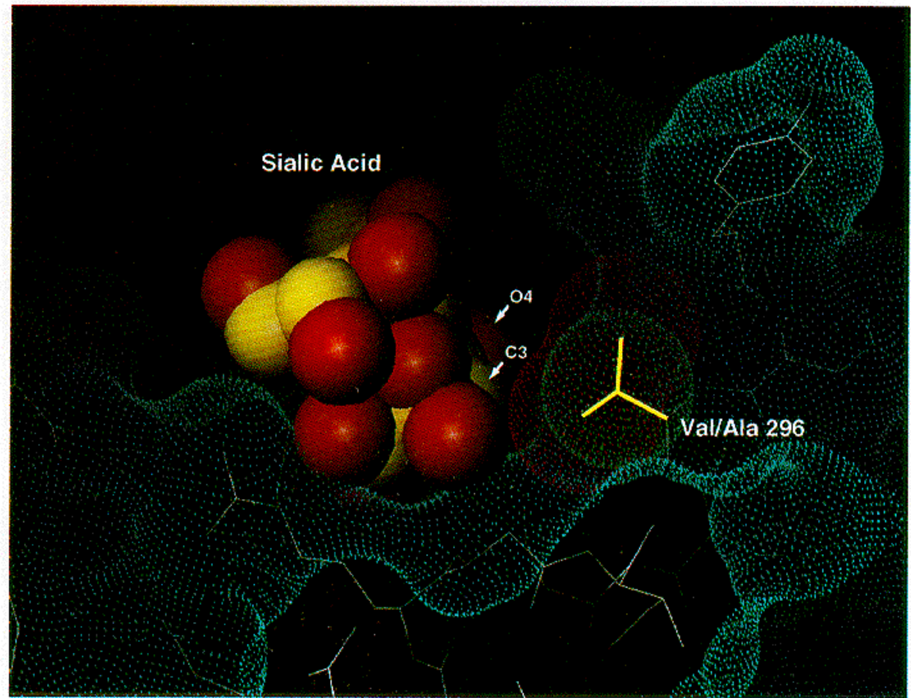
Sialic Acid
O4
C3
Val/Ala 296