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Comparison of the Deduced Amino Acid Sequence of Guinea Pig Adenovirus Hexon Protein with That of Other Mastadenoviruses

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Purpose: **We sought to isolate, clone, and determine the nucleic acid sequence of the guinea pig adenovirus (GPAdV) hexon gene. From this, the amino acid sequence of the cloned portion was deduced and compared with a set of mastadenovirus hexons.**

Methods: **The DNA isolated from a histologic section of infected guinea pig lung was subjected to high-fidelity amplification, using degenerate primers complementary to a conserved nucleic acid sequence near the 3' end of the hexon gene of mastadenoviruses and a 5' primer from GenBank accession No. X95630 (GPAdV hexon gene partial sequence). The amplified product was cloned, the nucleic acid sequence was determined, and the amino acid sequence was deduced and compared with the hexon amino acid sequences of 25 mastadenoviruses.**

Results: **The cloned fragment comprised 1,603 base pairs (bp) [~50%]) of the hexon. Of the initial 278 nucleic acids of the clone, 276 were identical with GenBank accession No. X95630, and the deduced amino acid sequences of both were identical. The deduced GPAdV hexon amino acid sequence from the clone aligned with structural regions NT, V1, DE1, and FG1 described for human adenovirus types 2 and 5. The GPAdV hexon had < 50% similarity in amino acid sequence, compared with hexons of 25 other mastadenoviruses. Analysis of regional peptide similarities revealed the GPAdV hexon to be more similar to animal mastadenoviruses and human subgroups A, C and F than to other human subgroups.**

Conclusions: **The cloned portion of the GPAdV hexon contained a sequence nearly identical to that of GenBank accession No. X95630. Compared with the truncated amino acid sequences of human adenovirus types 2 and 5, the deduced GPAdV hexon amino acid sequence was similar in areas structurally conserved, but different in areas associated with type-specific antigenicity.**

Adenovirus as an infective cause of bronchial pneumonia in guinea pigs was first reported in Germany (1) and later in North America (2, 3), Australia (4), and several European countries (5-7). Initial descriptions of this disease were that of lethal adenoviral pneumonia of guinea pigs. The presence of subclinical disease associated with this virus was suspected in those reports; however, supportive serologic and histologic evidence was lacking (1-4). Kunstyr and co-workers (8) were able to reproduce the disease in newborn guinea pigs. To date an in vitro method has not been found to support sufficient propagation of this virus to allow isolation of genomic DNA for molecular biological analysis. Antigens derived from other mastadenoviruses of animals and humans have failed to reliably detect antibodies produced in guinea pigs on response to presence of this virus (8).

A polymerase chain reaction (PCR) was developed to a 281-base pair (bp) portion of the gene encoding the hexon coat protein of the guinea pig adenovirus (9). An abbreviated form of this PCR was incorporated with histologic and serologic methods to study the pathogenesis of the guinea pig adenovirus in the respiratory tract (nasal turbinate mucosa) of guinea pigs (10). This latter study documented that the life cycle of this virus appeared to be transient replication in the upper respiratory tract mucosa for up to 15 days, causing an "occasional sneeze," with no observed signs of clinical or lethal pneumonia (10). Little else is known about guinea pig adenovirus (GPAdV) in relation to other adenoviruses in the genus *Mastadenovirus*.

In the study reported here, we isolated, cloned, and sequenced approximately 50% of the gene encoding the hexon coat protein of the GPAdV from preserved tissue obtained during a recent outbreak of this disease (11). The GPAdV nucleic acid sequence was used to deduce the amino acid sequence of the cloned portion of the hexon coat protein gene. The deduced amino acid sequence was compared with that of other mastadenoviruses.

Materials and Methods

Isolation of guinea pig adenovirus DNA. Deoxyribonucleic acid of the GPAdV was isolated from a paraffin-embedded histologic section of infected guinea pig lung documented to contain the virus in a recent epizootic (11). The section was deparaffinized in xylene at 50°C for one hour. The tissue on the slide was rehydrated through graded alcohols (100, 95, and 70%) for one hour each and placed in water for one hour. The tissue was removed from the slide by use of a clean razor blade, and the DNA was isolated, using a DNeasy kit (Qiagen, Valencia, Calif.) according to the manufacturer's recommendations for animal tissues. The isolated DNA was subjected to PCR analysis, using a proofreading thermostable DNA polymerase (Platinum *Pfx*, Life Technologies, Bethesda, Md.). The DNA concentration was determined by measuring absorbance at 260 nm; DNA purity was determined by examining the ratio of absorbances at 260 and 280 nm.

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Development of PCR primers. All nucleic acid sequences for alignment and comparative analyses were obtained from either the NIH GenBank or European Molecular Biology Laboratory (EMBL), or the Swiss Protein Database (SWISSPRO). The known GPAdV hexon gene sequence (accession No. X95630) was aligned with that of mouse adenovirus FL strain (MAD1, accession No. P48308), bovine adenovirus type 3 (accession No. P03278) and porcine adenovirus type 3 (accession No. AF83132), using Omiga 1.1 software (GCG/Oxford Molecular Group, Cambridge, UK). The alignment disclosed that the known sequence of the guinea pig hexon gene was near the 5' end of the gene. A PCR primer was defined at the 5' end beginning at the first ATG start codon for later experiments to determine protein expression. The sequence of this primer is ATGCACATCGCCGGCCAG. To identify a consensus sequence at the 3'end, a sequence alignment was performed, using hexon gene sequences from MAD1, human type 4 (accession No. AF065062), human type 7 (accession No. AF053085), human type 3 (accession No. S39298), human type 40 (accession No. P11819), and human type 41 (accession No. P11820). A degenerate reverse primer was identified with the consensus sequence AAGBWCCACTCRTARGTGTA (degeneracy = 16).

High-fidelity PCR analysis. One-hundred nanograms of the isolated DNA was subjected to PCR analysis, using a master mix containing 1 U of *Pfx* polymerase, 1X Platinum *Pfx* buffer, 0.5 μ*M* of each primer species, 200 m*M* of each nucleotide triphosphate and 1.5 m*M* $MgSO_4$. The amplification was performed on a Robocycler 9600 thermal cycler (Stratagene, La Jolla, Calif.) set for 40 cycles, with the following parameters: 94°C for 45 sec., 60°C for one minute, and 68°C for 2.5 min. This was followed by a final extension at 68°C for ten minutes for one cycle. The reaction product was analyzed by use of 1% agarose (Life Technologies) gel electrophoresis in 1X TRIS acetate EDTA buffer containing 0.5 μg of ethidium bromide/ml run at 90 volts for 30 min, and the results were photographed.

Cloning of PCR product. The PCR product was purified from the agarose gel, using a Gel Extraction kit (Qiagen) according to manufacturer's recommendations. The isolated fragment was cloned into pCR-Blunt II TOPO (Invitrogen, Carlsbad, Calif.) and transfected into chemically competent Top 10 *Escherichia coli* (Invitrogen) according to the manufacturer's instructions. Recombinant clones were selected on Luria Bertani (LB) agar (Life Technologies) containing kanamycin (50 μg/ml). Recombinant clones were identified by increased molecular size, compared with that of the parent plasmid, when analyzed by use of agarose gel electrophoresis. Clones were further analyzed by use of a *Pvu* II restriction digest (NEBiolabs, Beverly, Mass.) to identify the orientation of the insert (data not shown). Plasmid preparations of two clones in opposite orientations were grown in LB broth containing kanamycin (50 μg/ml). Both plasmids were isolated, using Plasmid mini-kit (Qiagen). The purity and concentration of the plasmids was validated by use of UV spectrophotometry (see GPAdV DNA isolation). These plasmids were submitted to the University of Virginia Biomolecular Research Facility for nucleic acid sequence determination, and the sequences of the two plasmids were compared by use of PRETTY software (GCG/Oxford Molecular Group, Cambridge, UK) sequence alignment.

Nucleic acid and amino acid sequence analyses. The cloned DNA sequence was analyzed, using various programs within the SeqWeb package of computer analysis software (GCG/Oxford Molecular Group). Initially the nucleic acid sequence was aligned with GenBank accession No. X95630 (9) to confirm the identity of the cloned fragment, using the aforementioned PRETTY software. The amino acid sequence of the cloned fragment was deduced using TRANSLATE software (GCG/Oxford Molecular Group). The deduced amino acid sequence was subjected to a BLAST search, using the NIH National Center for Biotechnology Information (NCBI) World Wide Web application; the results were represented, using TAXBLAST option to obtain GenBank, and SWISSPRO protein accessions with similar sequences of amino acids. The adenovirus hexon peptide sequences identified by the TAXBLAST search were imported and truncated to align with the C-terminal end of the deduced GPAdV amino acid sequence for further analysis.

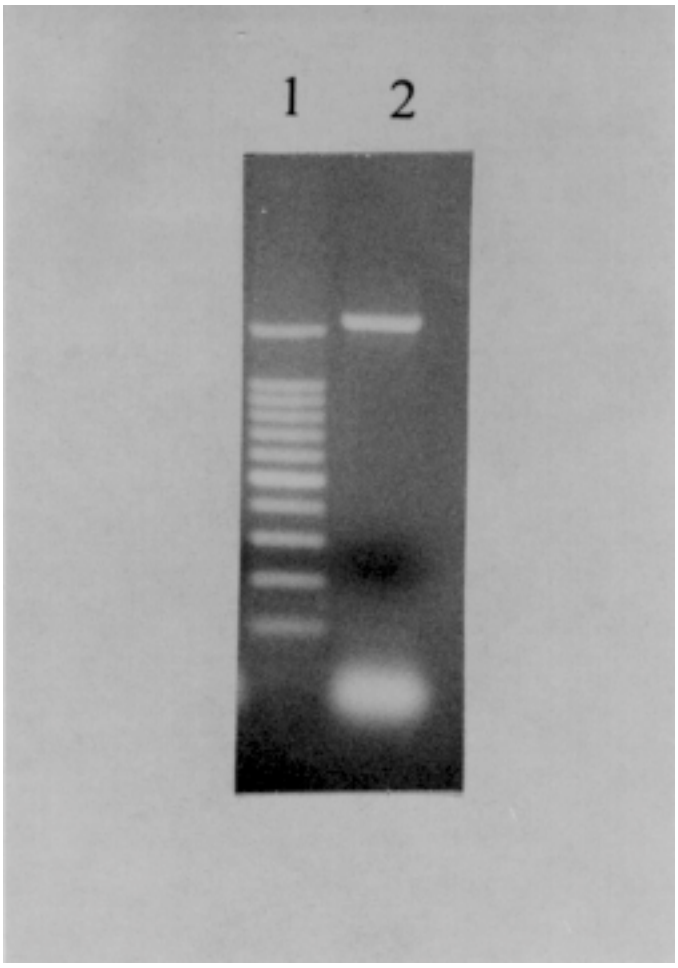

Figure 1. Photograph of an ethidium bromide-stained agarose gel (1%, 90 V for 40 min) showing polymerase chain reaction (PCR) production of an approximately 1,500-base pair (bp) amplicon, using the DNA isolated from infected tissue, as described in Materials and Methods. Lane 1 = 100-bp molecular size ladder, lane 2 = product of the PCR that was cloned.

To identify structural regions defined by the deduced amino acid sequence of the GPAdV hexon, a comparison was made with human adenovirus types 2 and 5. The hexon sequences of human adenovirus types 2 and 5 were truncated to the size of the deduced GPAdV amino acid sequence, and the three were subjected to a multiple sequence alignment, using the PRETTY software, for presentation with a consensus sequence.

To determine the similarity of the deduced amino acid sequence

```
          1                                                    50
  GPhexon ~~~-------- ---------- ---------- ---------- ----------
   X95630 ttc------- ---------- ---------- ---------- ----------
Consensus ~~~ATGCACA TCGCCGGCCA GGAGGCGGTA GACTACCTGT CTCCCGGCCT

          51                                                   100
  GPhexon ---------- ---------- -t-------- ---------- ----------
   X95630 ---------- ---------- -c-------- ---------- ----------
Consensus GGTGCAATTC GCCCGCGCCA C-GACAGTTA TTTCCATCTG GGCAATAAGT

          101                                                  150
  GPhexon ---------- ---------- ---------- ---------- ----------
   X95630 ---------- ---------- ---------- ---------- ----------
Consensus TTCGTAACCC CACCGTGGCA CCCACTCAAG AGGTCACGAC CGACCGCTCT

          151                                                  200
  GPhexon ---------- ---------- ---------- ---------- ----------
   X95630 ---------- ---------- ---------- ---------- ----------
Consensus CAGAGACTCC AACTGCGGTT TGTGCCTGTG GATCGGGAGG ACACTCAGTA

          201                                                  250
  GPhexon ---------- ---------- ---------- ---------- ----------
   X95630 ---------- ---------- ---------- ---------- ----------
Consensus TGCGTATAAG ACCCGTTTTC AACTGGCCGT GGGAGACAAT CGCGTCTTGG

          251                            281
  GPhexon ---------- ---------c ---------- -
   X95630 ---------- ---------t ---------- -
Consensus ACATGGGCAG CACGTACTT- GACATTCGCG G
```

Figure 2. The PRETTY software-determined nucleic sequence alignment of the initial 278 nucleotides of the hexon clone (GPhexon) and GenBank accession No. X95630 of guinea pig adenovirus (GPAdV) (9). Consensus sequence is shown at the bottom of each section. The two sequences differ at only two nucleotide positions (72 and 270). Symbol legend (~) is where no input sequence exists; (-) is where there is agreement of individual sequences with the consensus line. Lower case letters are shown for the individual sequences where there is no consensus.

of GPAdV hexon to that of other mastadenoviruses, a similarity matrix was established. Hexon amino acid sequences from GPAdV and 25 mastadenoviruses identified by the TAXBLAST search (NCBI) were analyzed, using the DISTANCES software (GCG/Oxford Molecular Group) incorporating the Kimura Protein Method option. Using the Kimura option, the similarity score is related only to the number of exact matches and gap positions are ignored. This similarity matrix was further analyzed by use of the GROWTREE software (GCG/Oxford Molecular Group) to produce a schematic tree-like schematic representation of mastadenovirus relatedness by comparing small peptide regions for similarity and using the neighbor joining method for minimization of overall branch length.

Results

The initial amplification reaction produced a product slightly > 1,500 bp when compared with a molecular size ladder (Fig. 1). Nucleic acid sequence determination of both clones yielded an identical consensus sequence of 1,603 bp. Comparison of the initial 278 bp of the cloned sequence with the 281 bp in GPAdV hexon accession No. X95630 indicated sequence consensus of all but two nucleotides, positions 72 and 270, of accession No. X95630 (Fig. 2). This confirmed the identity of the clones as arising from GPAdV in the isolated lung DNA. The deduced amino acid sequence of the cloned fragment consisted of a single open reading frame (+1) of 534 amino acids, beginning with the ATG codon in the 5' PCR primer and spanning the entire cloned fragment. At the two positions where the cloned nucleotide sequence differed from GenBank accession No. X95630, the deduced amino acid sequence did not differ. In GenBank accession No. X95630, nucleotide positions 70-72 ACC encode threonine as does the cloned nucleotide sequence ACT. Similarly, GenBank accession No. X95630 nucleotide positions 268-270 TTT encode phenylalanine as do the clone's nucleotide sequence of TTC. Two other open reading frames were examined as potentially encoding proteins of 258 amino acids (-3 frame nucleotides 774 through 1), 201 amino acids (-3 frame nucleotides 1,604 through 1,000) and 60 amino acids (+2 frame nucleotides 197 through 376). The BLAST software searches of GenBank, EMBL and SWISSPRO failed to identify accessions with amino acid sequences similar to those deduced from these other reading frames.

The TAXBLAST software input of the deduced amino acid sequence of the GPAdV clone retrieved GenBank and SWISSPRO accessions of hexon proteins from 25 mastadenoviruses with similar peptide amino acid sequence. The DISTANCES software produced a matrix of similarity (Table 1) that documented the GPAdV amino acid sequence to be < 50% similar to those of other adenoviruses retrieved by the TAXBLAST software search.

Table 1. Matrix of similarity generated by use of the deduced amino acid sequence of GPAdV hexon in comparison with that of 25 other adenoviruses determined to be most similar by use of the TAXBLAST (NCBI) search of the Gen Bank

Similarity Matrix Analysis	Bovine 7	Carpine 1	Human 11	Human 35	Human 34	Human 48	Human 8	Human 15	Human 19	Human 37	Human 2	Human 5	Human 16	Human 4	Human 3	Human 7	Human 40	Human 41	Human 12	Human 31	K9 - 1 (CLL)	K9 - 1 (RI261)	Equine 1	Bovine 3	Mouse 1	Guinea Pig
Bovine 7	100	74.3	7.66	7.8	11.05	7.36	13.56	11.60	11.27	8.93	2.19	8.76	12.46	15.56	10.73	14.33	17.05	17.07	15.48	10.50	13.91	13.91	13.18	17.85	12.53	17.12
Caprine 1		100	10.19	9.5	7.87	6.49	12.75	10.79	7.97	8.07	0.59	6.37	11.27	12.80	11.99	14.75	13.45	13.49	12.67	10.11	17.48	16.70	13.22	17.08	4.76	15.59
Human 11			100	90.2	82.18	70.18	70.19	68.71	68.46	71.63	55.37	54.48	74.17	69.27	72.23	75.39	62.02	61.03	59.97	56.64	46.64	46.64	45.13	40.74	33.17	45.01
Human 35				100	81.54	69.73	68.63	68.34	68.36	71.19	56.75	54.36	72.86	67.88	70.81	73.75	62.02	60.61	60.10	58.07	44.71	44.71	43.75	39.37	29.04	43.44
Human 34					100	69.92	69.18	70.75	69.28	71.10	54.12	52.77	73.47	68.90	70.01	72.34	62.06	63.93	60.55	58.51	42.57	42.57	41.41	39.46	28.75	45.33
Human 48						100	82.53	79.01	76.74	78.21	56.20	56.63	72.21	72.90	68.83	72.50	63.75	58.65	61.94	60.55	51.77	51.77	47.01	43.66	31.33	47.04
Human 8							100	78.90	77.37	78.03	56.06	55.92	69.91	70.90	68.19	72.97	61.77	62.71	58.71	61.31	50.89	50.89	46.60	45.19	33.01	48.72
Human 15								100	82.87	76.55	57.03	54.35	68.55	69.73	69.46	72.18	61.06	59.09	60.81	60.72	49.38	49.38	43.99	44.75	33.22	43.76
Human 19									100	77.19	52.74	50.72	70.97	71.07	66.36	70.37	62.26	58.22	60.94	59.83	47.87	47.87	41.89	39.87	28.36	43.44
Human 37										100	56.06	58.61	73.47	74.91	70.73	74.76	63.22	63.05	62.15	64.10	50.04	50.04	42.99	48.26	36.36	44.96
Human 2											100	73.41	56.76	57.46	54.24	58.98	58.06	58.19	57.64	56.93	42.25	42.79	36.34	32.86	28.99	39.05
Human 5												100	57.06	58.76	54.62	56.35	59.94	59.78	57.24	59.70	43.11	43.65	40.06	38.05	27.21	41.48
Human 16													100	92.63	70.34	71.76	63.25	63.37	63.19	62.52	49.04	49.04	45.56	40.61	33.44	43.96
Human 48														100	71.84	71.94	62.09	61.52	63.52	60.59	50.33	50.83	44.73	41.36	34.78	46.25
Human 3															100	89.62	59.88	58.76	59.38	60.00	46.15	46.15	42.45	41.79	31.45	40.74
Human 7																100	64.49	60.71	63.17	62.27	49.95	49.95	46.03	44.63	34.85	42.47
Human 40																	100	74.12	70.84	71.83	57.52	57.99	50.09	49.95	39.82	46.12
Human 41																		100	61.93	67.34	53.10	53.10	50.65	47.61	38.32	46.72
Human 12																			100	75.99	53.28	53.28	52.16	50.37	34.12	48.65
Human 31																				100	47.80	47.80	46.69	48.01	34.12	41.00
K9-1 (CLL)																					100	99.75	64.79	50.73	40.38	47.27
K9-1 (RI261)																						100	64.39	50.73	40.38	47.27
Equine 1																							100	47.78	32.92	44.42
Bovine 3																								100	34.30	44.83
Mouse 1																									100	29.22
Guinea Pig																										100

The amino acid sequences of all adenoviruses in this comparison have been truncated at the conserved region YEWS prior to constructing the matrix.

The GPAdV amino acid sequence was most similar to that of human adenoviruses types 8 (48.72%) and 12 (48.65%), and least similar to that of caprine type 1 (15.59%) and bovine type 7 (17.12%). The MAD1, often used as antigen for detection of antibodies to GPAdV in guinea pigs, was 29.22% similar to the GPAdV deduced amino acid sequence.

Amino acid sequence alignment of the deduced GPAdV hexon amino acid sequence with that of the truncated hexon amino acid sequences of human adenovirus types 2 and 5 are shown in Fig. 3. X-Ray crystallographic analysis of the structure of hexon protein from these two human adenoviruses has been reported (12, 13). The amino acid sequence alignment indicates that the cloned portion of the GPAdV hexon gene corresponds to that portion of the protein designated as the NT, V1, DE1, and FG1 structural regions in these two human adenoviruses (Fig. 3). The region known as V1 in human adenovirus type 5 contains a highly conserved structural motif known as the "viral jellyroll," which is found in the capsid proteins of many spherical viruses. The V1 region in human adenovirus type 5 consists of eight β-barrels formed by amino acid residues 64-107, 326-429, and 554-617 and shown schematically in Fig. 3 (13). These three stretches of amino acids indicate consensus with GPAdV deduced sequence of 83, 86.5, and 86.5%, respectively, the latter region having been truncated at residue 606 to correspond to the end of the cloned GPAdV fragment.

The GCG GROWTREE software analysis was performed to ascertain the relatedness of hexon peptide regions of the mastadenoviruses identified by the TAXBLAST software when compared with that deduced for the GPAdV hexon. The result of this analysis is depicted in the tree diagram shown in Fig. 4.

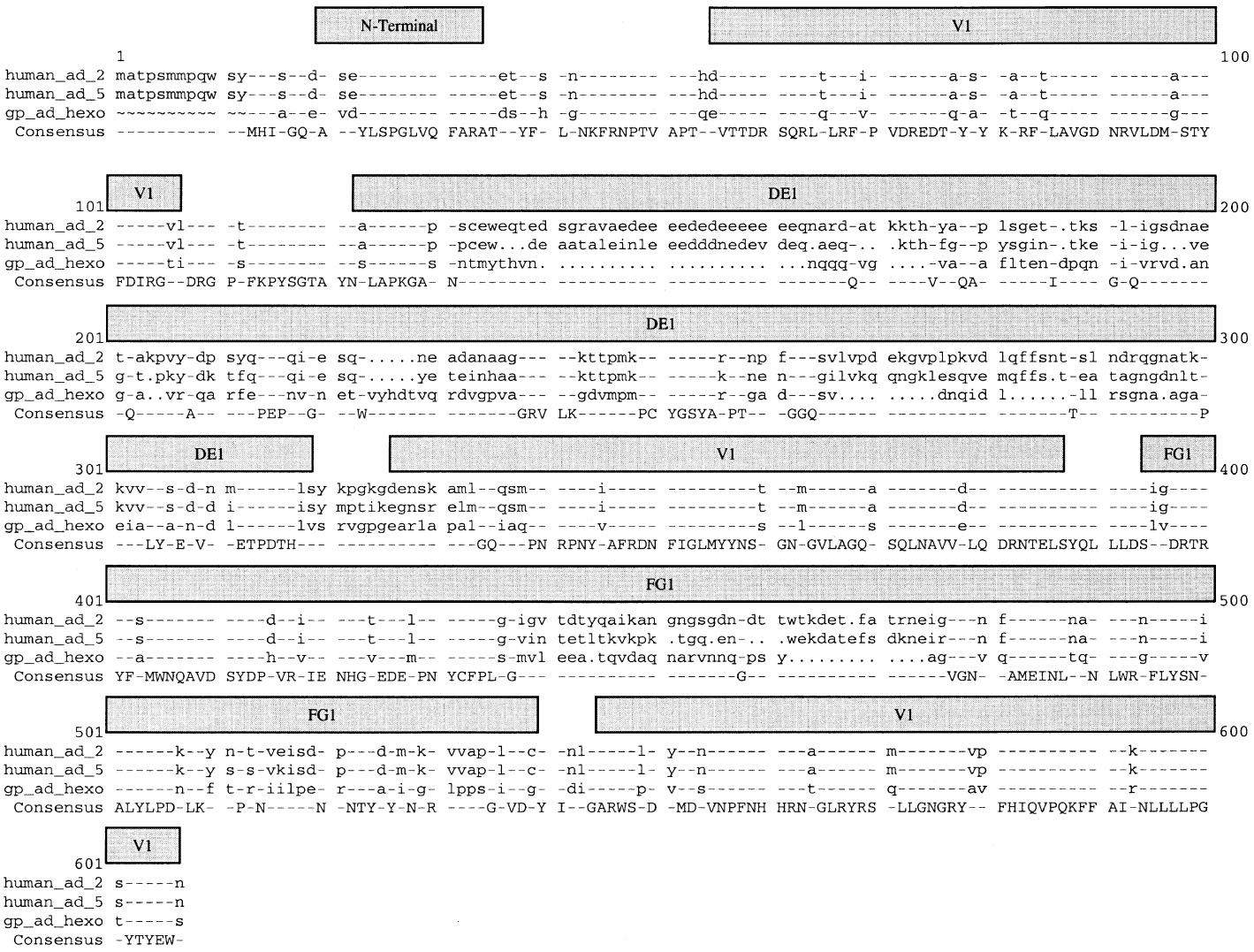

Figure 3. The PRETTY software-determined amino acid sequence alignment deduced for the hexon clone (GPAdV hexo) and human adenovirus types 2 (human ad 2, SWISSPRO accession HEX_ADE02) and 5 (human ad 5, SWISSPRO accession HEX_ADE05). The human adenovirus hexon sequences are truncated at the carboxy-terminal to coincide with the end of the deduced sequence of the GPAdV hexon fragment cloned. Where the three sequences are in consensus, an upper case letter is used in the consensus line and (-) indicates where there is no consensus. For the individual amino acid sequences, the symbol legend (~) is where no input sequence exists, (-) is where there is agreement of individual sequences with the consensus line, and (·) indicates where a gap is made in the individual sequence to facilitate the alignment procedure. Lower case letters are shown in the individual amino acid sequences where there is no consensus. The shaded bars indicate the topologic regions that correspond with the amino acid sequence according to references 12 and 13.

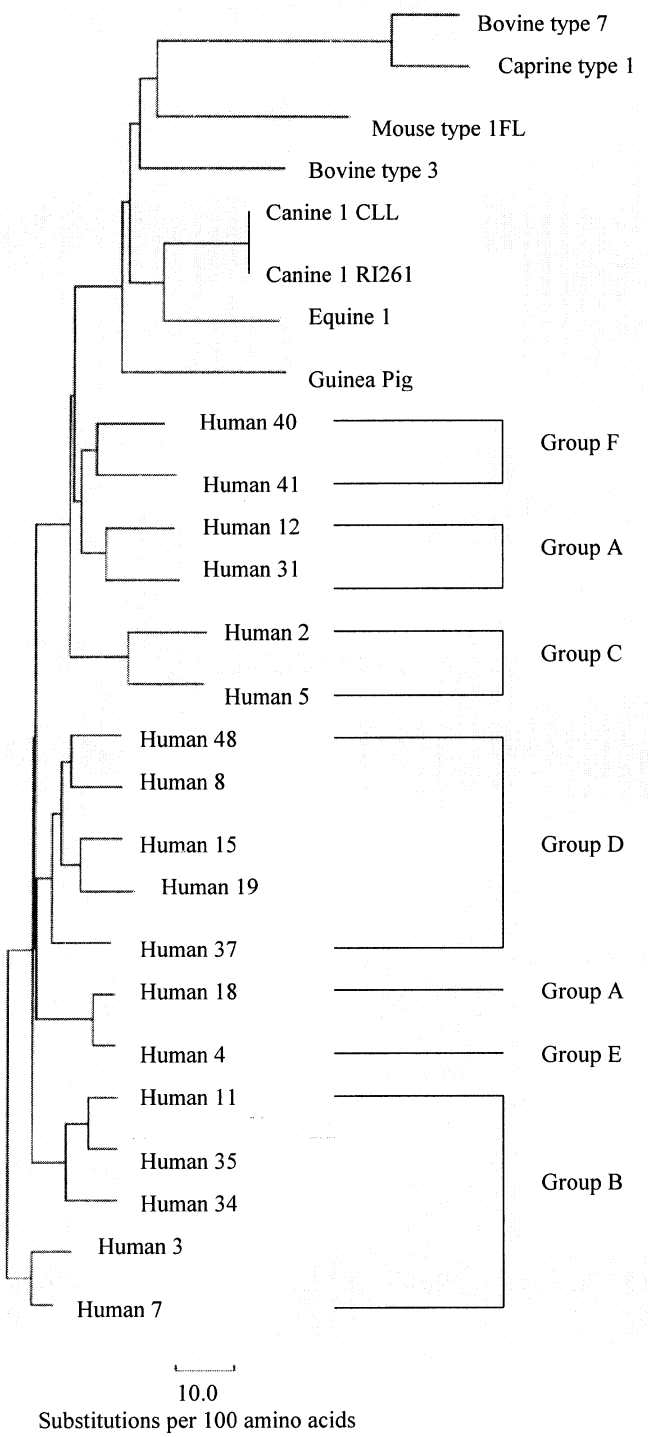

Figure 4. The peptide sequence relationships generated by GROWTREE software, using the truncated hexon amino acid sequences of the mastadenoviruses shown. The summation of horizontal branch lengths of the tree is proportional to the number of amino acid substitutions between two adenoviruses being compared. The clustering of adenoviruses to form a common branch is related to the number of consensus peptide fragments shared by the members within the cluster. (Size bar is equal to ten amino acid substitutions per 100 amino acids).

Bootstrapping was not performed, and so the tree is not rooted to any ancestral sequence. The vertical distance in this diagram depicts clustering of sequences that indicate regions of polypeptide similarities. The horizontal branch length in the tree diagram is related to the number of amino acid substitutions outside the areas of common polypeptide sequence. This analysis groups the GPAdV hexon with that of other animal mastadenoviruses and those of human subgroups A, C, and F, indicating that they share the greatest number of similar peptide fragments distinct from those of human subgroups B, D, and E. The animal mastadenoviruses form a subgroup distinct from human subgroups A, C, and F.

Discussion

The self-assembly of viral capsid is dependent on areas of protein-protein interaction between the component coat proteins. Peptide regions within the individual coat proteins involved in capsid assembly likely contain amino acids of highly conserved sequence, as sequence variation would likely preclude capsid assembly and subsequent viral replication. Proceeding on this premise, we assumed there would be areas of conserved peptide sequence within the hexon and that this would convey to conservation of the underlying DNA gene sequence. Alignment of hexon gene sequences from several mastadenoviruses identified 3' regions of conserved DNA sequence that were subsequently used in the development of a single long PCR encompassing > 50% of the GPAdV hexon gene. This method allowed us to overcome the difficulty in studying GPAdV due to lack of a purified source of genomic nucleic acid. The deduced amino acid sequence of the conserved 3' region of the hexon gene, which we used to generate our PCR fragment, corresponds with a region near the carboxyl-end of the V1 "viral jelly roll" (13). The conserved segmented regions of nucleic acid sequence encoding V1 flanked the nucleic acid sequences encoding the DE1 and FG1 regions of the hexon. The highly variable regions (HVRs 1 through 7 [13]) of structural entities DE1 and FG1 are associated with adenovirus type-specific antigenic determinants.

The GPAdV has proven difficult to study due to lack of an in vitro method of propagating the virus. Investigators have attempted to use antigens derived from other mastadenoviruses to study the incidence and/or development of antibodies in guinea pigs inoculated with GPAdV. Those investigators have reported lack of, or weak cross-reactivity, of guinea pig convalescent sera when examined, using heterotypic antigens (8, 9). Lack of homotypic viral antigen for use in detection of type-specific antibody detection further hampered studies (1, 9). Antigen derived from MAD 1 and 2 (K87) have been used most often in attempts to detect antibodies to GPAdV (9). Indeed, convalescent serum antibodies from guinea pigs purposefully infected with GPAdV recognized MAD 1 and 2 antigens by use of the indirect fluorescent antibody method only when studied at low (or no) dilution (10). We determined that there is a 29.22% similarity in amino acid sequence between GPAdV and MAD 1 in the region investigated, and predict that a somewhat better antigenic preparation for detection of anti-GPAdV antibodies might be human adenovirus type 12.

Most of the capsid mass of adenovirus is composed of three structural proteins, hexon, penton base, and fiber. The surface of the icosahedral shaped adenoviruses is composed of 240 hexon molecules in 20 capsid faces. At the 12 vertices of the icosahedron are penton base molecules attached to fiber proteins, together referred to as penton (13). The hexon and penton proteins of adenoviruses are the antigenic type-specific determinants of the capsid (14). Analyses of hexon residues, using anti-peptide sera

that can neutralize adenovirus infectivity in type-specific manner, suggest that the DE1 and FG1 regions are the antigenic determinants that are bound by these type-specific antibodies (13). In the study reported here, we cloned the nucleic acid sequence of GPAdV whose deduced amino acid sequence aligns with these type-specific regions of human adenovirus types 2 and 5. In our current work, we seek to use a mammalian expression system to obtain sufficient quantities of the cloned GPAdV truncated hexon protein to determine whether it can act as a suitable antigen for development of a GPAdV-specific enzyme-linked immunosorbent assay.

Analysis of the relationship of GPAdV to other adenoviruses was performed solely on the basis of putative hexon amino acid sequence. Analysis of this parameter alone correctly separated mastadenoviruses into subgroups wherein animal adenoviruses formed one subgroup distinct from several human adenoviral clusters. The clustering of human adenoviruses corresponded closely with adenovirus subgroups A through F, a grouping reflected in genomic nucleic acid sequence homology (15). It is noteworthy that a single human adenovirus (type 18) grouped incorrectly of the 18 human adenoviruses analyzed in the current study. Only truncated hexon amino acid sequence was used for the analysis of peptide relatedness. Human adenovirus 18 (part of group A) incorrectly clustered with human adenovirus type 4 (the sole member of group E). Results of this same analysis documented that GPAdV is a unique entity, compared with other members of the mastadenovirus genus.

Further investigation of GPAdV is warranted in part to develop reliable methods of serologic monitoring of this pathogen in colonies of laboratory guinea pigs. Therefore, cloning, analysis, and expression of major antigenic determinants of GPAdV may permit development of serologic tests that should assist in identification and eradication of this pathogenic and potentially lethal virus from laboratory colonies.

Acknowledgments

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