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Molecular Cloning and Sequence Comparison of the S1 Glycoprotein of the Gray and JMK Strains of Avian Infectious Bronchitis Virus

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Abstract. The nucleotide sequences of S1 glycoprotein genes of the Gray and JMK strains of avian infectious bronchitis virus (IBV) were determined and compared with published sequences for IBV. The IBV Gray and JMK strains had 99% nucleotide sequence similarity. The overall nucleotide sequence similarity of the Gray and JMK strains compared with other IBV strains was between 82.0% and 87.4%. The similarity of the predicted amino acid sequence for the S1 glycoproteins of the Gray and JMK strains was 98.8%. Six of the 10 differences in the amino acid sequence were found between residues 99 and 127, suggesting a possible role for that region in the tissue trophisms of the viruses. The S1 glycoprotein of the Gray and JMK strains had 79.5%–84.6% amino acid similarity with the published sequence of other IBV strains. Serine instead of phenylalanine was observed in the protease cleavage site between the S1 and S2 glycoprotein subunits for the Gray and JMK strains, which was similar to the published sequence for the Ark99 and SE17 strains. The significance of that amino acid change is not known. Based on the nucleotide sequence of the Gray and JMK strains, the *Bsm*AI restriction enzyme was selected by computer analysis and was used in restriction fragment length polymorphism analysis to differentiate the two strains.

Key words: infectious bronchitis virus, spike glycoprotein, coronavirus, cDNA, nephropathogenicity, polymerase chain reaction

Introduction

Avian infectious bronchitis virus (IBV) causes an acute, highly contagious disease of the respiratory and sometimes the urogenital tracts of chickens. Infectious bronchitis (IB) is an economically important disease to the poultry industry, and outbreaks continue to occur because different IBV serotypes do not completely cross-protect (1).

The virus is the type species of the family Coronaviridae, and its genome consists of one molecule of positive sense single-stranded RNA (2). It has three major structural proteins: a nucleocapsid protein, an integral membrane glycoprotein, and a spike (S) glycoprotein (3,4). The S glycoprotein is cleaved into N-terminal S1 and C-terminal S2 subunits (5,6). The S1 glycoprotein forms the distal, bulbous part of the S glycoprotein, and the S2 glycoprotein anchors the S glycoprotein to the membrane of the virion (7,8). Neutralizing, hemagglutination-inhibiting, and serotype-specific antibodies are directed

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The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers GRAYS1 = L14069 and JMKS1 = L14070.

against the S1 glycoprotein (9–12). Tissue tropism has also been associated with the S1 glycoprotein (13).

The S glycoprotein gene of several serotypes of IBV has been sequenced to investigate the antigenic variation of IBV at the molecular level (14–18). An amino acid sequence comparison of the Massachusetts 41 (Mass41) vaccine strain and the Beaudette laboratory strain revealed that S1 had two hypervariable regions (HVRs) (17). Antigenic and serotypic determinants of IBV are thought to be located in the HVRs (3,16,19).

Recently we reported on a polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) procedure to distinguish between serotypes of IBV (20). In that procedure three restriction enzymes (RE) were used to distinguish all of the known serotypes within the United States, as well as variant viruses. Only the Gray and JMK strains could not be differentiated from each other. In an attempt to distinguish between the Gray and JMK strains, over 23 RE were tested unsuccessfully. Serology indicates that the Gray and JMK strains are closely related and belong to the JMK serotype (21). The Gray strain, however, is nephropathogenic (22,23), whereas nephrotropism has not been reported for the JMK strain.

The objectives of the present study were to clone and sequence the S1 glycoprotein gene of the Gray and JMK strains of IBV in order to identify an RE that would differentiate the two strains in the PCR/RFLP serotype identification test. It is important to differentiate the two strains in a diagnostic test because the Gray strain is nephropathogenic. In addition, it is useful to know the sequence of serologically similar viruses that have differences in their tissue tropism. With that information we can begin to identify regions in the viral genome that may be associated with pathogenicity.

Materials and Methods

Viruses

Dr. Jack Gelb, Jr. (University of Delaware, Newark, DE) provided one Gray strain (22) chicken embryo passage 10 and two (received at different times) JMK strains (23), chicken embryo passage number 11. Another Gray strain (22), chicken embryo passage 9, was obtained from Dr. Pedro Villegas (University of Georgia, Athens, GA). All were passaged once in embryonating chicken eggs.

Viral RNA Purification

The viral RNA was extracted and purified as previously described (20). Briefly, sodium dodecylsulfate (final concentration, 2% wt/vol) and proteinase K (final concentration, 250 μg/ml) were added to allantoic fluid, incubated for 5 min at 55°C, and extracted with acid phenol and chloroform/isoamyl alcohol. The RNA solution was further purified using the RNaid™ kit (BIO 101) according to the manufacturer's recommendation, then stored at −70°C until used in the reverse transcriptase (RT) reaction.

Synthesis of cDNA by PCR

The S1OLIGO5′ and S1OLIGO3′ primers for the RT reaction and PCR, synthesized by the University of Georgia Molecular Genetics Facility, have been described previously (20). The sequence of the primers and their relative position in relationship to the S1 glycoprotein gene are shown in Fig. 1.

All of the reagents for the RT reaction and PCR have been described previously (20). Reverse transcripiton of RNA purified from allantoic fluid was done with Moloney murine leukemia virus reverse transcriptase (GibcoBRL) and primer S1OLIGO3′, which is complementary to a region at the 5′ end of the S2 glycoprotein gene. For the PCR reaction, the primer S1OLIGO5′, which is identical to a sequence near the 5′ end of the S1 glycoprotein gene, and 5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) were added to the RT reaction. For 35 cycles at 94°C for 1 min, 45°C for 2 min, and 74°C for 5 min, PCR was performed in a TwinBlock™ thermal cycler (Ericomp). The PCR products were electrophoresed (100 V constant voltage) on a 1% agrose gel containing ethidium bromide (0.5 μg/ml).

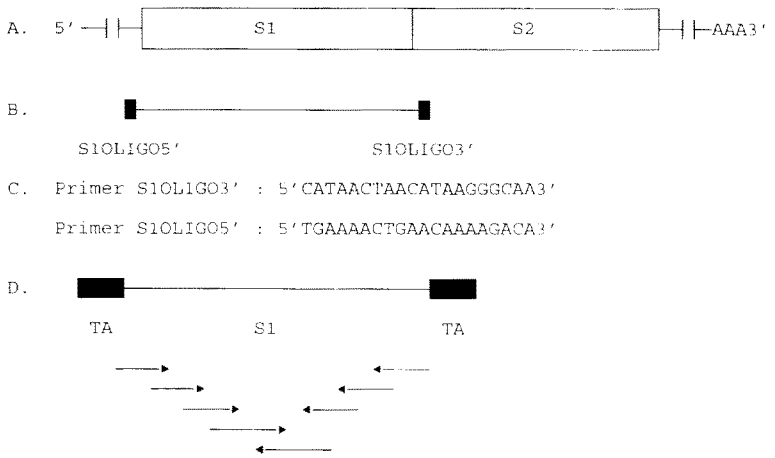

Fig. 1. Sequence strategy for cDNA of the S1 glycoprotein gene of IBV Gray and JMK strains. (*A*): IBV genomic RNA; boxes represent coding region of the spike glycoprotein. S1, N-terminal, and S2, C-terminal, cleavage products are indicated. (*B*): Relative annealing site of S1OLIGO3' and S1OLIGO5' primers and region amplified by PCR. (*C*): Primer sequences. (*D*): *Eco*RI digested PCR-amplified S1 glycoprotein gene cloned into the TA cloning vector. Arrows indicate the direction and extent of sequences obtained by selected primers.

cDNA Cloning

The S1 band, with a predicted size of approximately 1.7 kbp, was cut from an agarose gel and purified using the Geneclean kit (BIO 101) according to the manufacturer's recommendations. The purified DNA was ligated into the pCR™ II (Invitrogen Corp.) cloning vector, then transformed into competent *Escherichia coli* cells ($INV_{\alpha}F'$, Invitrogen). The white colonies carrying recombinant plasmids were selected from Luria-Bertani (LB) agar (24) plates containing kanamycin (50 μg/ml) and 25 μl of 40 mg/ml X-gal stock solution. The alkaline lysis method was used for small preparations (mini-preps) of plasmid DNA. The purified plasmid DNAs were digested with *Eco*RI (Promega) and analyzed on a 1% agrose gel to determine the size of the insert. Cesium chloride density gradient centrifugation was used to obtain larger amounts of plasmid DNA for sequencing.

DNA Sequencing and Sequence Analysis

Denatured double-stranded cloned DNA was sequenced by the dideoxy chain termination procedure using the Sequenase version 2.0 kit (USB) as recommended by the manufacturer. Initially, the M13 forward (USB) and reverse (#1201) primers were used for sequencing. In addition, six other primers were synthesized to various regions within the Gray strain of IBV (Fig. 1). At least three clones of each strain were sequenced. Nucleotide sequence data were compiled and analyzed on a IBM personal computer using the PC/GENE software (IntelliGenetics, Inc.).

RFLP Analysis

The S1 PCR products of the IBV Gray and JMK strains were purified on an agrose gel as previously described (20) and were digested with *Bsm*AI (NEB, Beverly, MA) according to the manufacturer's recommendations. The restriction fragment patterns were observed following electrophoresis (100 V constant voltage) on a 2% agrose gel containing 0.5 μg/ml ethidium bromide.

Results

Comparison of Nucleotide and Amino Acid Sequences

The nucleotide sequence of the entire S1 portion of the S glycoprotein gene, including the signal sequence for the Gray and JMK strains, is shown in Fig. 2. A comparison of the amino acid sequences deduced from the nucleotide sequences

```
Gra  TGAAAACTGAACAAAAGACAGACTTAGTTCTTAATTTAATTAAGTGTGGT-50
JMK
Bea                              CT
M41                              CT
A99                        C     AG        C A G AAC
SE1  ************************************************
PP1  ******                      CT

Gra  AAGCTACTGGTAAGAGATGTCGGTACGACCTCTTTTGCTAGTGACGTGTT-100
JMK
Bea     T              T    AC          A         TCT
M41     T              T    AC          A         TCT
A99   AT GT A  T       T   GAAGT A  G   T         CAT
SE1  ***********       T   GAAGT A  G   T         CAT
PP1     T G            T    AC          AT        TCT

Gra  TGTTAGCACTAGGTAGTGCTAGTTTGCTTAATAATGATTCTTATGTGTAC-150
JMK
Bea     GT      T       GT     TA G C G AG       C  T
M41     GT T    T       GC     TA G C G AG       C  T
A99      T      T        A    ATA G C  C  A     T      T
SE1      TT     T        A    ATA G C                 T
PP1      T      T       GC     TA G C   AC       C  T

Gra  TACTACCAAAGCGCCTTCAGACCACCTAATGGTTGGCATTTACATGGAGG-200
JMK                                          C
Bea            T                 G                A  G
M41            T     T                            C  G
A99         G  T  T  T  G   GGAC
SE1         G  T  T  T  G   GGACC
PP1            T  T  T  G   GGAC

Gra  GGCTTATAAAGTAGTTAATGTTTCTGAGGAATATAATAATGCACCTGGCA-250
JMK
Bea  T      GCG         CA     AGC    T          GGCTCTT
M41  T      GCG          A     AGC    C          GGCTCTT
A99  T      GC             G   AGT   A          GG ACTG
SE1                           C     CT          GG CATG
PP1  T      GC             G  G     GCT          GG AC T

Gra  ATTCTGGTTGTGTGGCTGGTGCCATTTTTTGGAGCAAGAATTTTAGTGCT-300
JMK                                          C
Bea  CA  A  G   ACT T    ATT   CA G TG TCGTGT G   A
M41  CAC    G   A T T    A T   CA G TG TCGTGT G   A
A99  CCC AA    CACT         T   GGC AC  T        C     G
SE1  CACAG A    CGT         T  CGGC AC  T      C
PP1  CCCAAA     A T         T   GGC AC  T        C     G

Gra  GCTTCTGTAGCCATGATTGCACCACATAGTGGTATGTCGTGGTCTGTCCA-350
JMK
Bea  T     A    T    CG     GTCATCA      G T      AG AG
M41  T     A    T    CG     GTCATCA      G T      AG AG
A99    C  A          C       TA           A       C TC
SE1  T       G  G    C       T                   AACTA
PP1    C  A          C       CA           A       C TT

Gra  GCAATTTTGCACGGCTCACTGTAATTTTACTCATTTTACAGTGTTTGTTA-400
JMK
Bea  T  G     T  T  A            T AG  AC
M41  T  G     T  T  A         C  T AG  AC
A99  ATCT     T  A                  TC  A  T
SE1  A      A T           C        CT
PP1  ATCC     T  A  A              CTC  A  T
```

Fig. 2. Nucleotide sequence of the S1 glycoprotein gene region of the IBV Gray (Gra) strain, showing differences in the JMK, Massachusetts 41 (23), and Beaudette (1), Ark99 (A99), SE17 (SE1), and PP14 (PP1) (29) S1 genes. The S1OLIGO5′ and S1OLIGO3′ primers for RT and PCR are underlined. Dashes were introduced to align the sequences. Asterisks indicate unavailable sequences. Double underlining indicates nucleotide sequence encoding a connecting peptide of spike precursor polypeptide. Boldface type indicates a signal sequence including the ATG start codon for the spike glycoprotein gene. A dotted background indicates recognition sites of *Bsm*AI.

```
Gra  CACATTGTTTTAAGGTAGGAGCTGGTCTTTGTCCTTTAACAGGTCCCCTT-450
JMK                C                                 T A
Bea          A   ACAT  T GGT   C  TAA  GGC TGCT  AA AG
M41          A   ATAT AT GGT   C ATAA  GGC TGC   AAAAG
A99               AGC   T  AA AG         G        TTA
SE1               AGC   TT  CA AA         G       TTTA
PP1               AGC   C  AA AC         G        TTA

Gra  TTACAAGGCCAAATCCGCATTTCTGCTATGAGAAGCGTTAATAGTCGTCC-500
JMK  CC A                                             T
Bea  AAT TTATA GTG TTCT------------------ C  TG AAAA GG
M41  AATTTTTTA GTG TTCT------------------ C  TG AAAA GG
A99  CC AGC  TT T  T  T   G          A CAT GA G C  ACG
SE1  CA TCA  TTCT  T      G               GATG  A AACG
PP1  CC AAC  TT T  T  T   G          A CAT GA G GT AA

Gra  TCATC---TTTTTTATAATTTAACAGTTTCTGTGACTAAATATCCTTTATTTA-550
JMK       ---
Bea  C  G ---    C               AG   AG    G  C   ACT
M41  C  G ---    C               AG   AG    G  C   ACT
A99   GG  ACT A        C                             AAG
SE1   TC TGTA A               T G     T A   T       AC
PP1   GG TACT A        C                             AAG

Gra  AGTCACTCCAATGTGTTAATAATCAAACGTCTGTATATTTAAATGGTGAT-600
JMK
Bea  GA   T T  G            TT   A  C
M41   A   T T  G            TT   A  C
A99  GA  G  A                T  T                     C
SE1      G  A               TTT  A     G  C           C
PP1  GA  G  A                T  T                     C

Gra  CTTGTCTTTTCATCTAATGAGACTATAGATGTTTCAGGTGCAGGTGTTCA-650
JMK
Bea       T ACA C           C         A  TC          T
M41       T ACA C           C C       A  TC          T
A99       T  CA       CT T   GA       GT  C         C
SE1       T    A      C  C            AGT           T
PP1       T  CA C     CT T   GA       GT  C         C

Gra  TTTTAAAGCTGGTGGACCCATAACTTATAAAGTTATGAAGGAAACCAAAG-700
JMK
Bea                   T                    GA   GTT
M41                   T                    GA   GTT
A99         AG        T                    GA  GGTT
SE1                             C
PP1         AG        TG                   GA  GGTT

Gra  CTCTGGCTTATTTTGTTAATGGTACTGCACAAGATGTTATTCTTTGTGAT-750
JMK
Bea   C                                        T G
M41   C                                        T G
A99   CT             C              T     C     A
SE1    T
PP1   CT             C              T     C     A

Gra  GGTTCACCTAGAGGTTTGTTAGCATGTCAGTATAACACTGGTAACTTTTC-800
JMK
Bea    A           C           C        T     C  T
M41    A           C           C        T     C  T
A99   ACA                      C  A     T     C  T
SE1           T                          T
PP1   ACA                      C  A     T     C  T

Gra  AGATGGTTTCTATCCTTTTACTAATGGTACTATTGTTAAGGATAGGTTTA-850
JMK                            C
Bea       C  T               A   G T A      C G A
M41       C  T          T    A   G T A      C G A
A99       C                  AC  G              A
SE1        C                 TA
PP1       C                  AC  G              A
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Fig. 2 (Continued).

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Gra  TTGTTTATCGTGAAACTAGTGTCAATACTACTTTGACACTAACTAATTTC-900
JMK
Bea      C           A       T           GT  GT  CAC
M41      C           A       T            T  GT  CAC
A99                  G                       T
SE1                  A                      GT   A
PP1                  G  T                    T

Gra  ACGTTTCGTAATGAAAGTGCCGCTCCTCCTAATAACGGTGGTGTCGACAC-950
JMK
Bea   TT    A       G C  G   CAAC       CCTA        TC G A
M41    T    A       G C  G   CAAC       CCTA        TC G A
A99       A              GT  C           CA         T    G
SE1       TT   CC        AT  C T         GT         T  T
PP1       A              GT  C           CA         T    G

Gra  TTTTATCTTATATCAGACGCAAACAGCTCAGAATGGCTATTATAATTTTA-1000
JMK
Bea   A  CAAACT  C  A  AA             G   T
M41   A  CAAACT  C  A  A              G   T
A99       T     C     A              G   T
SE1      G T            A             G   T
PP1       T     C     A              G   T

Gra  ATTTTTCATTTCTGAGTGATTTTGTCTACAAGGCATCTGATTTTATGTAT-1050
JMK                           T
Bea         C          AG       T  T    AG   A
M41         C          AG       T  T    AG   A
A99                    AG       T  T G  A AG A   A
SE1         C          AG       T  T    AG          C
PP1                    AG       T  T G  A AG A   A

Gra  GGATCTTACCACCCACATTGTAGTTTTAGACCAGAGACCCTTAATAATGG-1100
JMK          T
Bea          T       AG      AA        T   A  TA
M41          T       AG      A         T   A  TA
A99             T  GGC                  T  A      ---
SE1               T                     T  A  T
PP1             T    G                   T  A

Gra  TTTGTGGTTTAATTCACTTTCAGTTTCAATTACTTACGGTCCTCTTCAAG-1150
JMK
Bea  C                                  G
M41  C                                  G
A99          C       C     T       T A TA          CA
SE1                  C   A T       C A  A          CA
PP1                  T     T  A    T A  A           A

Gra  GTGGTTGCAAGCAATCTGTCTTTAGTGGTAGAGCAACTTGTTGTTATGCT-1200
JMK
Bea                           AA
M41
A99          T            A    A      A
SE1          T
PP1                           A      A       AA AA

Gra  TACTCATATAGAGGTCCTTCGCTGTGTAAAGGTGTTTATTCAGGTGAGTT-1250
JMK                      T
Bea    T       G
M41    T       G
A99    T      CG     A   CGTGCT            C   AG         C
SE1           C              T
PP1    T      CG T         CGTGGT            C   AG         C

Gra  ACTTCGTAATTTTGAATGTGGACTGTTAGTTTATGTTACTAAGAGCGATG-1300
JMK
Bea   GA  A                                                G
M41   GA  T
A99   ACA AGC               TT
SE1    G  A
PP1   ACA AGT               TT                        A
```

Fig. 2 (Continued).

```
Gra  GCTCTCGTATACAAACAGCCACTGAACAGCCAGTTATAACTCAATACAAT-1350
JMK
Bea                              C                 A
M41                              C               G C
A99      C           T  A  AC    CA  T  AT     C   A TTT
SE1      C     G                 CT                C
PP1      C           T  A  AC    CA  T  AT     C   A TTT

Gra  TATAATAATATTACTTTAAATACTTGTGTTGATTATAATATATATGGCAG-1400
JMK
Bea
M41
A99          C  C       GG  AG                G T      T
SE1                     G
PP1          C          GG  A                 G T      T

Gra  AACTGGCCAAGGTTTTATTACTAATGTAACCGACTTAGCTGTTAGTTATA-1450
JMK    T           C
Bea                              G        C
M41                                       C
A99        A                        T  T       AC TCCC
SE1        T
PP1        A                        T  T       AC TC C

Gra  ATTATTTATCAGACGCAGGTTTGGCTATTTTAGATACATCTGGTGCCATA-1500
JMK
Bea       C  G                                        T
M41       C  G                                        T
A99      C   G G  G G   A  A
SE1         G
PP1         G G  T     A  A

Gra  GACATCTTCGTTGTACAAGGTGAATATGGTCCTAACTATTATAAGGTTAA-1550
JMK
Bea         T                      T   T
M41         T                      T  CT
A99                              C        C
SE1         T                 C  C
PP1                       C    C  C        C

Gra  TCCATGTGAAGATGTCAACCAACAGTTTGTAGTTTCTGGTGGTAAATTAG-1600
JMK                              C
Bea  C  T  C              G
M41  C  T  C              G
A99    T            T
SE1                 T
PP1    T            T

Gra  TAGGTATTCTCACTTCACGTAATGAAACAGATTCTCAGCTTCTTGAGAAC-1650
JMK                              G
Bea            T              G  T G
M41            T              G  T G
A99                              T G         C       A
SE1                              T G                 A
PP1            T                 T G         C       A

Gra  CAGTTTTATATTAAAATCACTAATGGAACTCGTCGTTCTAGACGTTCTGT-1700
JMK
Bea          C  C                  A       T          A
M41          C                     A       T          A
A99          C     G               A A
SE1          C                   GTA A                A
PP1                G             TT ATA     T

Gra  TACTGAAAATGTTACAAATTGCCCTTATGTTAGTTATG-1738
JMK
Bea              G
M41              G
A99    A           G
SE1                T
PP1    A  G         G                  AA
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Fig. 2 (Continued).

of the Gray and JMK strains is shown in Fig. 3. Also included in Figs. 2 and 3 is a comparison with published sequences (14,17,18).

The IBV Gray and JMK strains had similar S1 sequences. The Gray and JMK strains differed by only 1% (17/1738) in their nucleotide sequences. The Gray and JMK strains had between 82.0% and 87.4% nucleotide identity with the Mass41, Beaudette, Ark99, SE17, and PP14 strains. The Gray and Ark99 strains had the least similarity, and the Gray and SE17 strains had the most. The Gray and JMK strains had 18 extra nucleotides at a position 469–486 (Fig. 2) that were not found in the nucleotide sequences of the Mass41 and Beaudette strains.

The Gray and JMK strains differed by 1.2% (10/557) in their amino acid sequences. Most of the differences in the amino acid sequence were found between residues 60 and 127. A highly variable region containing six differences was observed between residues 99 and 127. The Gray and JMK strains had between 79.5% and 84.6% amino acid identity with the Mass41, Beaudette, Ark99, SE17, and PP14 strains of IBV. A dendrogram of the amino acid alignment is presented (Fig. 4). The Gray and JMK strains had the least similarity to Mass41, and the most similarity to the SE17 strain. Like Ark99 and SE17, the Gray and JMK strains had a serine (residue 523) instead of phenylalanine in the cleavage site of the connecting peptide between the S1 and S2 glycoproteins (Fig. 3).

RFLP Analysis

Based on a computer RE analysis of the nucleotide sequence for the Gray and JMK strains, the *Bsm*AI RE was selected for use in the RFLP analysis of the two strains. Following digestion of the PCR product with *Bsm*AI and electrophoresis, the Gray and JMK strains had the expected restriction fragment patterns (Fig. 5), which could be used to differentiate between them.

Discussion

The purpose of sequencing the S1 glycoprotein genes of the Gray and JMK strains of IBV was twofold. First, we wanted to identify a RE for use in our PCR/RFLP serotype identification test that would distinguish between those viruses. Second, we wanted to add the sequence of those strains to the growing database of S1 glycoprotein sequences for strains of IBV in the United States. Those data are a first step toward identi-

```
   -18                 +1
    |                  |
Gra  MSVRPLLLVTCLLALGSASLLNNDSYVYYYQSAFRPPNGWHLHGGAYKVV-32
JMK
Bea   L T      L C  C  V YDSS             S    Q    A
M41   L T      L CV C  A YDSS                       A
A99   L KS F   I F  C  N YD E F          GH         A
SE1   L KS F   I FS C  N YD              GP
PP1   L T      L F  C  A YD T            GH         A

Gra  NVSEEYNNAPGNSGCVAGAIFWSKNFSAASVAMIAPHSGMSWSVQQFCTA-82
JMK                                 L
Bea   I S F   GSS   TV I HGGRVVN S I  T  S   A  SS
M41   I S S   GSSP  IV T HGGRVVN S I  T  S   A  SS
A99     S N   GTAPS T    GY           T  L      ASS
SE1     Q L   GHAQD R    GY       S   T  L      TK  S
PP1       L   GTSQS I    GY           T  P      ALS

Gra  HCNFTHFTVFVTHCFKVGAGLCPLTGPLLQGQIRISAMRSV-NSRPHLFYN-132
JMK                   A         LIPK           -   L
Bea      SDT        Y H G--      M Q NL  V   KNG-----Q
M41      SDT        Y YDG--  I   MRQKNFL V   KNG-----Q
A99       SY          S SNS     LIPS Y   A  KHGSRTPG
SE1       Y           S SHN     FIQS S   A    GC NASCI
PP1       SYI         S PNT     LIPN Y   A  KHGSVNPGY
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Fig. 3. Amino acid sequence of S1 glycoprotein of the IBV Gray (Gra) strain, showing differences in the JMK, Massachusetts 41 (23), Beaudette (1), Ark99 (A99), SE17 (SE1), and PP14 (PP1) (29) S1 genes. Asterisks indicate unavailable sequences. To conform to other published sequences for S1, numbering begins after the signal sequence (boldface). Dashes were introduced to align the sequences. The double-underlined sequence is a connecting peptide of the spike precursor polypeptide.

```
Gra  LTVSVTKYPLFKSLQCVNNQTSVYLNGDLVFSSNETIDVSGAGVHFKAGG-182
JMK
Bea       A   T R        L           YT        TS   Y
M41       A   T R F      L           YT     T  TS   Y
A99           K R        H            T  Y E  VA       S
SE1   L  NN   T          F            T  D           Y
PP1           K R        H            T  Y E  VA       S

Gra  PITYKVMKETKALAYFVNGTAQDVILCDGSPRGLLACQYNTGNFSDGFYP-232
JMK
Bea         R V
M41         R V
A99         R V            H       DT
SE1                                   I             L
PP1  V      R V            H       DT

Gra  FTNGTIVKDRFIVYRETSVNTTLTLTNFTFRNESAAPPNNGGVDTFILYQ-282
JMK
Bea     SSL  QK       N      C  H  I H  TG N  PS  QNIQL
M41  I SSL  QK       N      F  H     H  TG N  PS  QNIQT
A99     TS    K       S                S   G    T    S
SE1     Y             N          N     F Q D L  S      V
PP1     TS    K       SI               S   G    T    S

Gra  TQTAQNGYYNFNFSFLSDFVYKASDFMYGSYHPHCSFRPETLNNGLWFNS-332
JMK
Bea   K   S            S     E N         S K  L  I
M41       S            S     E N         S N  L  I
A99       S            S    RE NY        A          -    S
SE1       S            S     E    I      L
PP1       S            S    RE NY        R

Gra  LSVSITYGPLQGGCKQSVFSGRATCCYAYSYRGPSLCKGVYSGELLRNFE-382
JMK
Bea       A              K             G               DH
M41       A                            G               DL
A99     LI    I          N K           G   RA      R   TQH
SE1   T  L    I                                        R
PP1     L    I          N K   RS      G   RG      R   TQY

Gra  CGLLVYVTKSDGSRIQTATEQPVITQYNYNNITLNTCVDYNIYGRTGQGF-432
JMK                                                  I   L
Bea            G           P      N
M41            G           P     RH
A99                       QP   L  NF        GK      V
SE1                 M       P      H        D
PP1           R           QP   L  NF        GN      V

Gra  ITNVTDLAVSYNYLSDAGLAILDTSGAIDIFVVQGEYGPNYYKVNPCEDV-482
JMK
Bea        S        A             S             L
M41        S        A             S             LT
A99          T H    AEG                                 L
SE1                 A
PP1          T H    A                       Q           L

Gra  NQQFVVSGGKLVGILTSRNETDSQLLENQFYIKITNGTRRSRRSVTENVT-532
JMK                           G
Bea                           G                    F   I    A
M41                           G                    F   I    A
A99                           G   P              H       N
SE1                           G                  VH      I
PP1                           G   P              SY F     NG

Gra  NCPYVSY-539
JMK
Bea
M41
A99
SE1        *
PP1       RN
```

Fig. 3 (Continued).

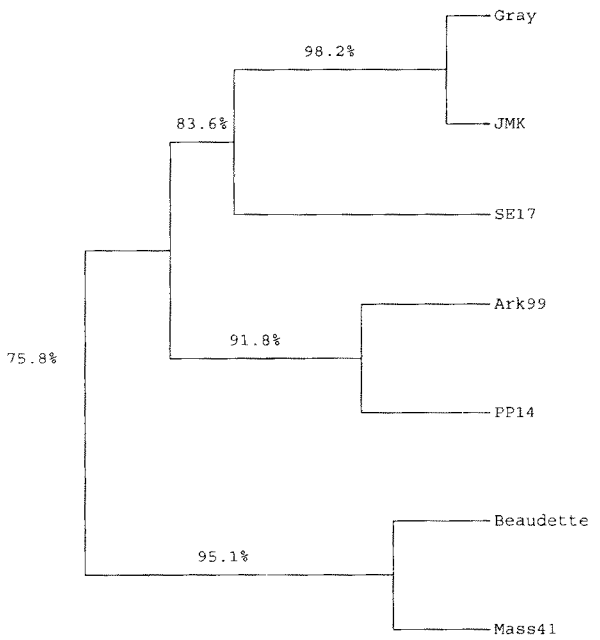

Fig. 4. Genomic relatedness based on the amino acid sequence of the S1 glycoprotein for Gray and JMK strains of IBV, compared with published sequences for five other strains of IBV.

fying neutralizing and serotype-specific epitopes, and regions that are involved in attachment of the virus to target cells. The S1 glycoprotein sequences of Gray and JMK presented here are the first published sequences for this serogroup (designated JMK).

By computer search and agarose gel electrophoresis, the *Bsm*AI was found to be the best enzyme for distinguishing between the Gray and JMK strains in our PCR/RFLP serotype identification test. Three restriction sites were observed in the JMK strain at bases 445 (within HVR2), 613, and 1078; the Gray strain had two sites at bases 613 and 1078.

Ten differences in the amino acid sequences of the S1 glycoprotein were observed between the Gray and JMK strains. Beaudette and Mass41 (both Massachusetts serotypes) are reported to have 26 differences in their amino acid sequences (15). Six of the 10 differences between the amino acid sequences of the Gray and JMK viruses were in a variable region between residues 99 and 127. This corresponds to a variable region with the Massachusetts serotype reported by Niesters et al. (17) between residues 117 and 131.

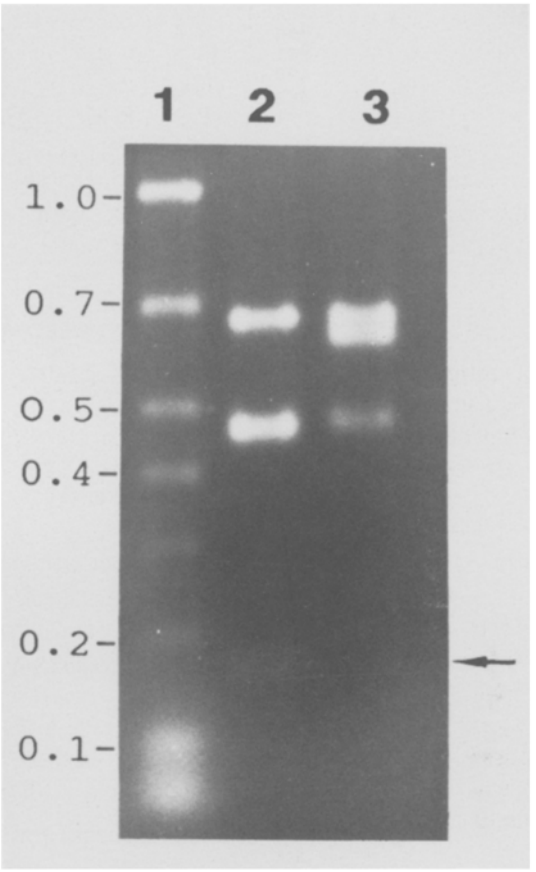

Fig. 5. The RFLP patterns of the PCR-amplified S1 glycoprotein genes from IBV Gray and JMK strains digested with *Bsm*AI. Lane 1 = molecular-weight marker Biomarker (BioVenture, Inc., Murfreesboro, Tenn.); lane 2 = JMK; lane 3 = Gray. Numbers at the left are molecular weight markers in kilobase pairs. The arrow indicates the band at the bottom of lane 2.

The overall differences in the amino acid sequences observed between all of the IBV strains examined herein were located between residues 34 and 138 and 234 and 324. Similarly variable regions between residues 40 and 129 and 271 and 378 have been reported by Cavanagh et al. (19) for closely related serotypes of IBV. Our data extend this observation to include different serotypes of IBV, suggesting (as others have) that these regions may be involved in forming serotype-specific and virus-neutralizing epitopes.

A protease cleavage site between the S1 and S2 glycoprotein subunits was reported to be Arg-Arg-Phe-Arg-Arg for the Beaudette and Mass41

viruses (5,13). The cleavage site of the Gray and JMK strains was similar to the recently published sequence for Ark99 and SE17 (18), wherein a serine instead of a phenylalanine (residue 523) was observed. Although both amino acids are uncharged at physiological pH, serine has an aliphatic hydroxyl side chain, whereas phenylalanine has an aromatic side chain. The significance of this amino acid difference with regard to virulence is not known.

The Gray and JMK strains of IBV are the same serotype, indicating that they are very similar antigenically. However, the pathogenicity of these viruses is different because the Gray strain can produce a nephritis. It follows that the amino acids located between residues 99 and 127 may play a role in the different observed pathogeneses for these viruses. This observation is supported by Cavanagh et al. (13), who observed an amino acid difference within the HVR2 region of two vaccine viruses, which may account for the differences in virulence observed for those viruses. The molecular basis for tissue trophism may become more apparent as the sequence becomes available for other nephropathogenic strains, such as Holte (22), Australian T (26), and one of the Holland strains (22).

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