MOLECULAR BASIS OF THE VARIATION EXHIBITED BY AVIAN INFECTIOUS
BRONCHITIS CORONAVIRUS (IBV)

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ABSTRACT

IBV serotype-specific, virus neutralising (VN) antibody is induced by the S1 subunit of the spike (S) glycoprotein. A Portuguese isolate (Port/322/85) is considered to be an *in vivo* recombinant. VN tests showed that it belongs to the Massachusetts (Mass) serotype. Correspondingly, the sequence of S1 and S2 was extremely similar to that of older Mass isolates. However, the matrix (M) glycoprotein and upstream gene sequences were atypical of these Mass strains and more closely resembled other serotypes isolated in Europe. The recombination event had occurred within the sequence between the M and S genes (corresponding to mRNA 3) or near the 3' terminus of the S2 gene.

INTRODUCTION

It has been known for decades that IBV exhibits extensive antigenic variation. On the basis of VN tests isolates have been assigned to serotypes, the number of which continues to increase^{1,2}. Serotype-specific VN antibody is induced by S1, the amino-(N)-terminal half of the spike (S) glycoprotein³⁻⁶. Moreover, amino acid differences between isolates are concentrated within the N-terminal 25% of S1 residues⁶⁻⁸ and some epitopes for VN antibody are situated in this region⁶ (Fig.1).

Fig.1. Amino acid variation within S1 of 8 Mass serotype strains (from reference 6). Vertical lines indicate where at least one of seven Mass strains differed in sequence from the M41 strain. The arrow shows the position of an amino acid substitution in a mutant of M41 which resisted neutralization by a monoclonal antibody.⁶

In this communication we present evidence that a strain of the Massachusetts serotype is actually a recombinant.

METHODS

Isolate Portugal/322/85 was received in 1985 and was serotyped by virus neutralization (VN) tests in chicken tracheal organ cultures¹. IBV RNA was produced and sequenced essentially as described previously⁹.

RESULTS

Strains of the Mass serotype have always been a major cause of severe outbreaks of IB and the most widely used vaccines are of the Mass serotype. Our analysis of a number of vaccine and field strains, all of the Mass serotype but isolated over a four decade period, showed that S1 had been strongly conserved (S2 was not sequenced)⁶ (Fig.1). Taken as a group, the 7 strains analysed varied from the M41 strain, at only 6% of amino acids. The 3c ORF and M gene of the vaccinal strains had also been highly conserved⁹.

Isolate Port/322/85 was neutralised by antisera to several Mass strains including M41 and H120 and was considered to be of the Mass serotype. Sequencing of S1 gave data which correlated with the serological analysis. Thus the S1 protein of Port/322/85 shared 94.9% homology with that of M41 (Fig.2). Several of the amino acids possessed by Port/322/85 and different from M41 were identical to those in the vaccine strain H120, the field strain HVI-140 (Fig.2) and the other Mass strains previously sequenced⁶. However, the 3c ORF and M gene sequence of Port/322/85 was not at all like that of these other Mass strains (Fig.3). Rather, it more closely resembled the sequences of UK/6/82 and UK/200/83⁹, both in terms of deletions and the possession of specific nucleotides. We then sequenced the S2 part of the S gene. There was 97.4% amino acid homology with S2 of M41 (we determined 87% of the S2 sequence of Port/322/85). We were not able to determine the carboxy-(C)-terminal amino acids because of the complete failure of the oligonucleotide used to prime the reverse transcription of this part of the gene. The oligonucleotide was complementary to IBV-M41 sequence a few nucleotides downstream from the C-terminus of S2, in the 3a ORF.

Fig.2. Comparison of the S1 amino acid sequence of Mass serotype strains Port/322/85, H120 and HVI-140 and the non-Mass strain UK/6/82 with that of the M41 strain. Each vertical line represents an amino acid difference.

(a)

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M41      UGGUAGAAACUUAACAAUCCGGAUUAGAAGCAGUUAUUGUCAACGAGUUCCUAAGAACGGUUGGAUUAUAAAAUCCAGCAAUUUUCAAG
MM/Branch      U
H52/H120      U
Ibvax      U
322/85      A      A      G      A G      C      A      AC *****
UK/200/83 C      A      A      G G      A G      A      AC *****
UK/6/82  C      A      A      G      A G      .      A      A      A      AU*****

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(b)

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M41      AUGUCCAACGACACAAUUGUACUCUUGACUUUGAACAGUCAGUUGAGCUUUUAAAGAGUAUAU
MM/Branch
H52/H120      A
Ibvax      A
322/85      *****AU      A      UAC C G AGU C . .. C G U
UK/200/83 ***** UGACAGA      UAC      AG      C      C      G      A
UK/6/82      GAU      ACC      C      GUAC      AG      C      G      A

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(c)

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M41      UUAUUUUAAACUGCAUUCUUGUUGUUCUUAACCAUAAUACUUCAGUUGGCUAUGCAACAAGAAGUAAG
MM/Branch      .....
H52/H120      U
Ibvax      U
322/85      C . CG      G      C U      C      C      .      G      C      G      CCG
UK/200/83      C      C      C      UC U U      C U      A      C      U      G      CCGU
UK/6/82      C      G      C      C      C      A      C      U      G      AC

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(d)

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M41,MM,Branch,  MSNETNCTLDFEQSVELFKEYNLFITAFLLFLTIIILQGYATRSK
H52, H120,Ibvax
322/85      ***M      TQ VA. . D      V      L      R
UK/200/83      **MTE      T A Q      L      R
UK/6/82      D T      GT A Q      V      L      N

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Fig.3. M gene sequences. Only those nucleotides which differ from those of M41 are shown; unidentified nucleotides are marked with a dot (.). (a) sequences of the 3' end of the 3c ORF; (b) sequence coding for the exposed part of M; (c) sequence for the first membrane-embedded part of M. Nucleotides absent from some sequences are indicated by asterisks (*); (d) comparison of the amino acid sequences of the M protein corresponding to nucleotide sequences in (b) and (c).

The striking similarity of the S1 genes of Port/322/85 and M41 and equally striking dissimilarity of their 3c ORF and M sequences indicates that Port/322/85 is a recombinant. The recombination event would have occurred either within the gene 3 ORFs or, given the failure of the above-mentioned oligonucleotide, near the C-terminus of S2.

DISCUSSION

The results presented herein and the recent findings of Kusters et al.⁸ strongly support the view that some IBV isolates are naturally occurring recombinants. Given the high frequency of recombination which occurred during experimental mixed infections with murine hepatitis virus strains¹⁰ it seems likely that many IBV strains are recombinant at one or more loci.

Strains of the Mass serotype continue to be isolated, 50 years after the first Mass isolate was made. Given the widespread use of Mass serotype vaccines - some countries, including the UK, permit only live vaccines of this serotype - it might be assumed that a Mass isolate is a re-isolate of a vaccine strain. Sequencing of S1 can indicate if a Mass isolate is a vaccine strain, a mutant of a vaccine or a nonvaccine related Mass field strain (which from the data reported herein, some

isolates clearly are). However, given the phenomenon of recombination, such data can be no more than a tentative indication of the identity of an isolate. Sequencing of the M gene improves the situation but this still leaves unexamined more than 20 000 nucleotides, among which there may have been point mutations, deletions, insertions and/or recombination events!

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