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The Recognition of a vlhA Protein from the F-Strain of *Mycoplasma gallisepticum* with Monoclonal Antibody 6F10

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Abstract: The goal of this project is to identify the genes encoding *M. gallisepticum* F-strain surface proteins recognized by specific antibody reagents to characterize the individual role of each gene product in host colonization. Here we report the characterization of a 70-kDa surface protein recognized by monoclonal antibody 6F10. The surface protein was gel isolated and digested with trypsin for evaluation with mass spectrometry. The results showed that 19 out of the 21 tryptic peptides matched with proteins of the vlhA lipoprotein family. We suggest that monoclonal antibody 6F10 is specific for the predominantly expressed vlhA protein (formerly pMGA) of the F-strain of *M. gallisepticum*.

Key words: *Mycoplasma gallisepticum*, F-strain, monoclonal antibody 6F10, vlhA, mass spectrometry

Introduction

Virulent strains of *Mycoplasma gallisepticum* colonize epithelial surfaces in the upper respiratory tract in chickens and initiate a respiratory infection described as chronic respiratory disease (CRD). Typical of mycoplasmal diseases, the *M. gallisepticum* strains involved in CRD syndrome can persist for the life of an infected animal (Yoder, 1991). The virulent field strains of *M. gallisepticum* inflict significant economic losses on broilers, broiler breeders, commercial layer chickens, and turkeys (Mohammed *et al*., 1987).

Monoclonal antibodies (mAb) have been generated for use as diagnostic reagents for the various strains of *M. gallisepticum* found in poultry (May *et al*., 1994). Such reagents are particularly important in the differentiation of virulent field strains from the live vaccine strains used in poultry flocks. To this end, a number of hybridomas were raised against one of the most widely used vaccine strains, the F-strain of *M. gallisepticum* (termed F-strain for brevity) at the USDA/ARS South Central Poultry Research Unit (May *et al*., 1994). One of the hybridomas, termed 6F10, showed the strongest reactivity for the F-strain and was further characterized by a collaborating laboratory (Garcia and Kleven, 1994) as a candidate reagent for selectively identifying the F-strain in vaccinated chickens. In those studies, chickens were experimentally infected with a 6F10-positive culture of the F-strain cells, and then samples of the tracheal flora were examined at various times post-infection. These experiments revealed a lack of 6F10-surface expression on some of the clonal isolates obtained from the trachea, indicating that the protein recognized by this mAb was subject to variation in expression. Brown *et al*. (1997) used mAb 6F10 to study the re-isolation rate of the F-strain vaccine during an entire laying cycle of experimentally vaccinated hens. Their results were similar to the findings of Garcia and Kleven (1994) in that tracheal isolates were found to be heterogeneous in expression of the 6F10 epitope. Furthermore, some of the 6F10-negative isolates expressed the epitope after several passages *in vitro*, confirming that the expression of the gene contributing the 6F10 epitope may be regulated by phase variation. Western blot analysis of total proteins derived from various clonal isolates showed that the protein possessing the epitope recognized by mAb 6F10 also varied in molecular weight (70 to 80 kDa) from one isolate to another (Garcia and Kleven, 1994). Taken together, these studies indicated that the protein(s) expressing the epitope recognized by mAb 6F10 is subject to both variation in molecular weight (size variation) and variation in surface expression (phase variation). Similar properties have been identified for surface proteins from a number of mycoplasma species found in animals, which has led to the proposal that such mechanisms are utilized by mycoplasmas to avoid elimination by host immune responses (Reviewed in Citti and Rosengarten, 1997). Our long-term goal is to understand the mechanism(s) that are responsible for the F-strain's ability to prevent host colonization with virulent field strains. Therefore, our objectives are to identify and characterize the genes encoding F-strain surface proteins recognized by antibody reagents and to determine the individual role of each gene product in host colonization. Here we report the initial characterization of the surface protein recognized by mAb 6F10.

Materials and Methods

Mycoplasma proteins: The F-strain isolate was obtained from Dr. S. H. Kleven (University of Georgia) and was propagated in Frey's medium supplemented with 12% swine serum at 37°C in an anerobic chamber (Vardaman and Drott, 1978). Cells from 24-hour cultures of the F-strain were washed 3 times in phosphate buffered saline, and the membrane and cytosolic proteins were extracted with Triton X-114 as described previously (Wan *et al.*, 2004). The protein quantities of the whole cell lysate and the Triton X-114 aqueous (cytosolic proteins) and detergent phases (membrane proteins) were determined with the 2D-Quant protein assay reagents (Amersham Biosciences, San Francisco, CA, USA).

Western blotting: Mycoplasma proteins (5µg/well) were resolved on 0.7 mm thick 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) at 100V constant voltage for 2 hours using the Laemmli buffer system (Laemmli, 1970). To visualize proteins, gels were stained in 0.25% Coomassie brilliant blue and then destained in 5% methanol and 7.5% acetic acid. For Western blotting analysis, proteins were transferred to nitrocellulose membranes in transfer buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3) using a Mini Trans-Blot cell (BIO-RAD, CA, USA) at 100V constant voltage for 1 hour (Wan *et al.*, 2004). Proteins were then detected with mAb 6F10 by a modification of the procedure described by Avakian and Kleven (1990). Briefly, membranes were blocked overnight at 4°C in Tris buffered saline (TBS) containing 5% horse serum and 1% bovine serum albumin, followed by a room temperature incubation for 1 hour. Membranes were then washed 5 times with TBS-0.05% Tween 20 and then incubated with monoclonal antibody 6F10 diluted 1/100 in primary buffer (TBS containing 5% horse serum, 1% swine serum) for 1 hour at room temperature. Membranes were then washed 5 times with TBS-0.05% Tween 20, and then incubated with rabbit anti-mouse IgG-alkaline phosphatase conjugated (Sigma Chemical CO, USA) that was diluted 1/10,000 in secondary buffer (TBS containing 5% horse serum, 1% swine serum, and 1% rabbit serum) for 1 hour at room temperature. Membranes were washed as above and then developed with SigmaFast NBT/BCIP tablets (Sigma Chemical Co, USA) for 5 minutes.

Mass spectrometry: Protein bands were excised from Coomassie blue - stained 10% SDS-PAGE for digestion with trypsin as described (Collier *et al.*, 2006). Briefly, gel slices were destained, reduced with dithiothreitol, and then alkylated with iodoacetamide. All chemicals were HPLC grade from Sigma. Gel slices were then incubated overnight at 37°C in 25µl of 6ng trypsin/µl (Promega, WI, USA), followed by extraction of the tryptic peptides from gel slices by incubation in a mixture of 1% trifluoroacetic acid and 2% acetonitrile for 30 minutes at 37°C. Tryptic peptides were then desalted with MicroTrap C18 column (Michrom Bioresources, Inc, USA), vacuum dried, and then resuspended in 0.1% formic acid. Samples were then evaluated with a 1-dimensional liquid chromatography and LCQ Deca XP Plus mass spectrometer (ThermoElectron Corporation, San Jose, CA, USA) at the MSU Life Sciences and Biotechnology Institute Core facility.

Data analysis: The amino acid sequences of the tryptic peptides was determined by matching the mass spectrometry data with amino acid sequences derived from the non-redundant mycoplasma protein database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) using Bioworks Browser version 3.1 (ThermoElectron Corporation, San Jose, CA, USA) software as described previously (McCarthy *et al.,* 2005). The amino acid sequences of tryptic peptides were also evaluated using BLASTp searches against mycoplasma proteins in the non-redundant protein database http://www.ncbi.nlm.nih.gov/Entrez. Amino acid sequence alignments were conducted with the ClustalW version 1.7 program (Thompson *et al.*, 1994).

Results

Isolation of the protein recognized by mAb 6F10 from SDS-PAGE: Earlier studies used colony staining and flow cytometry to demonstrate that the protein recognized by mAb 6F10 is expressed on the surface of F-strain cells (Garcia and Kleven, 1994; Brown *et al.,* 1997). These studies were extended with experiments where F-strain cellular proteins were fractionated using Triton X-114. Western blot analysis of the Triton X-114 fractions revealed the 70-kDa protein recognized by mAb 6F10 in the whole cell lysate (Fig. 1a, lane 2), absent in the aqueous fraction (Fig. 1a, lane 3), and enriched in the detergent fraction (Fig. 1a, lane 4). Therefore, we applied the Triton X-114 detergent fraction to SDS-PAGE, and the 70-kDa protein was excised from Coomassie blue-stained gels (Fig. 1b) for trypsin digestion and analysis with mass spectrometry as described by Tu *et al.* (2005).

Identification of the protein recognized by mAb 6F10 with mass spectrometry: The results showed that 19 out of 21 tryptic peptides derived from the 70-kDa protein matched with products of the vlhA (formerly pMGA) lipoprotein family (Table 1). The two peptides not matching with vlhA proteins most likely represent contaminants of the 70-kDa protein excised from Coomassie-stained gels. With the recent completion of the *M. gallisepticum* R-strain genome sequence, the pMGA genes were renamed as vlhA for agreement with current nomenclature (Papazisi *et al.*, 2003). Therefore,

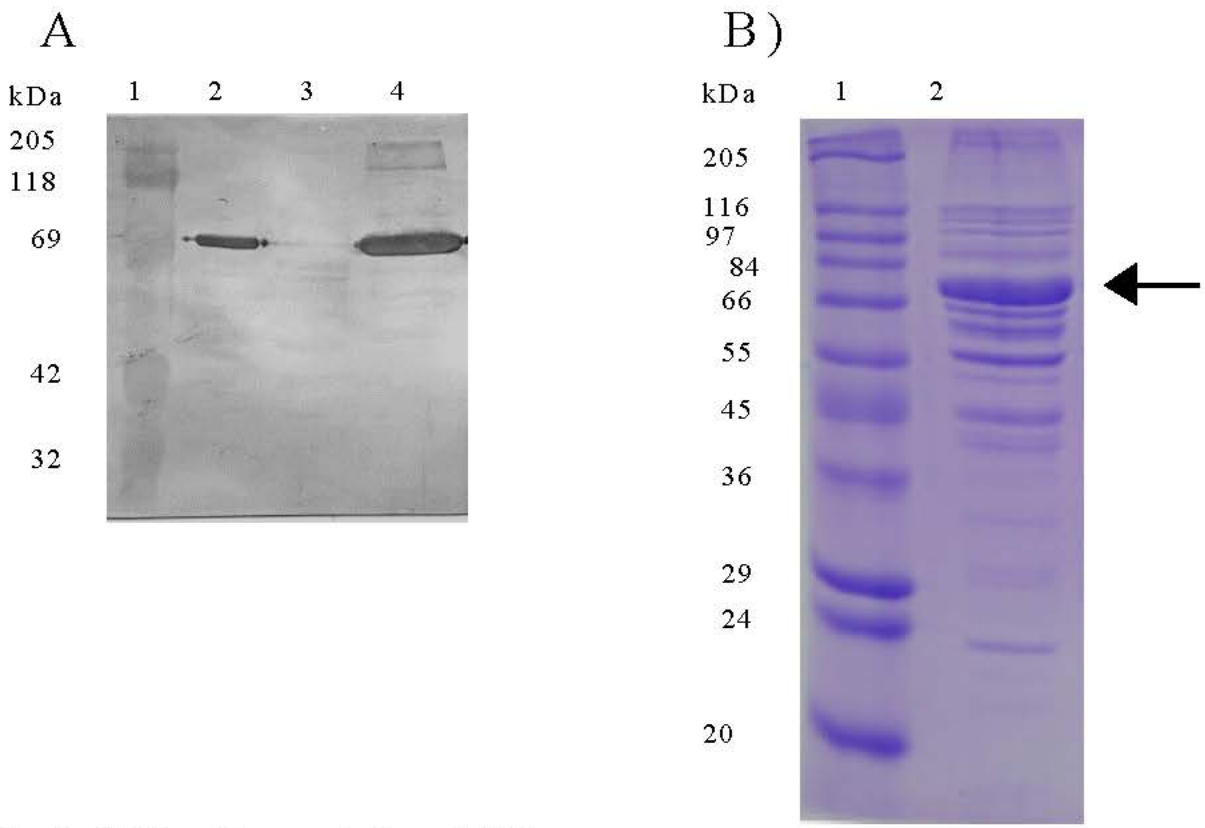

Fig. 1: The 70-kDa protein recognized by mAb 6F10.
A). Western blotting analysis of Triton X-114 partitioned *M. gallisepticum* F-strain proteins with mAb 6F10.
Lane 1. Molecular mass standard. Lane 2. Whole cell lysate. Lane 3. Aqueous phase. Lane 4. Detergent phase
B). SDS-PAGE of the Triton X-114 partitioned *M. gallisepticum* F-strain proteins.
Lane 1. Molecular mass standard Lane 2. Detergent phase
The 70-kDa band that was excised is indicated by an arrow.

the vlhA nomenclature will be used here except when referring to earlier reports using the pMGA nomenclature.

Analysis of vlhA-specific tryptic peptides: The Bioworks Browser analysis identified the predicted protein products from three vlhA genes: the vlhA.3.07 and vlhA.5.08 genes from the *M. gallisepticum* R-strain genome (Papazisi *et al.*, 2003) and the vlhA open reading frame (termed 9.3-orf) cloned from F-strain cDNA (Pharr *et al.*, 2002). The three-vlhA proteins identified in the database search are aligned in Fig. 2. The amino acid positions of the tryptic peptides within the proteins are given in Table 1 and shown underlined in Fig. 2. Only the sequence of one peptide, TALTSLLASK (amino acid positions 85- 94) is found in all three vlhA proteins (Fig. 2). Seven peptides show specificity for either the 9.3-orf protein or the vlhA.3.07 protein based on the 21 amino acid differences between the two proteins. Peptides NANIEMYSDYAK (positions 95-106), KETTVMTLKDPK (positions 174-185), and DLKFSTGDMSSNNVTIR (positions 574-591) align to regions of the 9.3-orf protein that differ from the vlhA.3.07 protein (Fig. 2). By contrast, peptides YSAILDQINGVSSK (positions 186-199), NITMVANTNQQR (positions 525-534), VTTVDTNRKTLTLVK (positions 615-629), and KTLTLVK (623-629) align to regions of the vlhA.3.07 protein that differ from the 9.3-orf protein. The remaining 11 vlhA-specific peptides aligned with regions that are conserved between the 9.3-orf and vlhA.3.07 proteins (Fig. 2).

Discussion

Markham *et al.* (1992) reported the preparation of mAb with specificity to surface expressed hemagglutinin proteins of the virulent *M. gallisepticum* S6 strain. The panel of mAb recognized a phase variable 67-kDa lipoprotein from the virulent field strains S6 and R, and a phase variable 75-kDa lipoprotein expressed by the F-strain. Additional functional studies showed that the mAb inhibited the hemagglutination of chicken erythrocytes by *M. gallisepticum* cells, suggesting that the mycoplasma protein was an adhesin gene product. The lipoproteins detected by the mAb were termed pMGA

```
                                    30                                60
                                    |                                 |
9.3             VKRKNILKFVSLLGIGSFVMVAAASCTSAATPTPNPEPN--PNPEPKPDPMPNPPSGGMN
vlhA.3.07       MKRKNILKFVSLLGIGSFVMLAAASCTSATTPTPNPEPKPTPNPEPKPDPMPNPPSGGMN
VlhA.5.08       MKRKNILKFVSLLGIGSFVMLAAASCTTPVNPTPNPTPN----PEPKPNPTPNPEPKPMP
                :********************:******:...***** *:    *****:* *** .  *

                                    90                               120
                                    |                                 |
9.3             GGDINPGGGQNMMDSAAQELTAARTALTSLLASKNANIEMYSDYAKIQNTLIAAYTTAEQ
vlhA.3.07       GGDINPGGGQNMMDSAAQELTAARTALTSLLASKNANVEMYSDYAKIQNTLIAAYTTAEQ
VlhA.5.08       ---N-PGGGGESMDNAAQELAAARTALTSLLASKNANVEMYSDYAKIKNDLTAAYTTAEQ
                     **** : **.*****:****************:*********:* * ********

                                   150                               180
                                    |                                 |
9.3             TSQNSSATLEQVKNATSALQTAINTANSNKQKFDQDHSNLLMSYKNLMATLAKKETTVMT
vlhA.3.07       TSQNSSATLEQVKNATSALQTAINTANSNKQKFDQDHSNLLMSYKNLMATLAKKETAVMT
VlhA.5.08       TSQNSSATLEQVKNATSTLQTAINTANSNKQKFDQDHSELLTSYKNLKTVVGKKDAVVME
                *****************:********************:** ***** :.:.**::.**

                                   210                               240
                                    |                                 |
9.3             LKDPKYSAILDQINGVSCKGEELVQHTLDPVSGIVPAANTITEEITKIEEVISEKTLQDQ
vlhA.3.07       LKDPKYSAILDQINGVSSKGEELVQHTLDPVSGIVPAANTITEEITKIEEVISEKTLQDQ
VlhA.5.08       LKDSKYSAILNEINSASLKAEKIIKETLDPISGEIPTSKMITDGTANIEKVINSETLMSQ
                ***.******::**..* *.*:::::.****:** :*::: **:  ::**:**..:** .*

                                   270                               300
                                    |                                 |
9.3             KNNADQFANYQSFTLDKTKLENVEDAKKMGQPANYSFVGYSVDVTGTS---GQETTIPNW
vlhA.3.07       KNNADQFANYQSFTLDKTKLENVEDAKKMGQPANYSFVGYSVDVTGTS---GQETTIPNW
VlhA.5.08       KENADQFANFKAYTIVKEKIMGTDDTHNQAQPANYSFVGYSVDVTGTTTAPGSETALPNW
                *:*******::::*: * *: ..:*::: .*****************:   *.**::***

                                   330                               360
                                    |                                 |
9.3             NFAQRAIFTSGNQPTKVTATTTGEDQSTAKPLSDVSWIYSLAGTGAKYTLEFTYYGPSTG
vlhA.3.07       NFAQRAIFTSGNQPTKVTATTTGEDQSTAKPLSDVSWIYSLAGTGAKYTLEFTYYGPSTG
VlhA.5.08       NYAQRTIFTSGDQPTKIDTPAAS-DETPAQPLSNVSWIYSLAGNGAKYTLDFTYYGPSTG
                *:***:*****:****: :.::. *:::.*:***:*********.******:*********

                                   390                               420
                                    |                                 |
9.3             WLYFPYKLVKANDDVGLQYKLNSNETLTPIIFGEGTTTNGPAATVENINVAKVRLTGLAF
vlhA.3.07       WLYFPYKLVKANDDVGLQYKLNSNETLTPIIFGEGTTTNGPAATVENINVAKVRLTGLAF
VlhA.5.08       YLYFPYKLVKNSDSVGLQYKLNNIPTETAITFGNMQNANGPTATVDSINIAKVTLSNLNF
                :********* .*.********.  * *.* **:  .:***:***:.**:*** *:.* *

                                   450                               480
                                    |                                 |
9.3             GKNTIEFSAPMSKVAPMIGNMYLTSSDTETNKQNIENSIFGNSVTTENNITKISVDTLSA
vlhA.3.07       GKNTIEFSVPMSKVAPMIGNMYITSSDTETNKQNIENSIFGNSVTTENNITKISVDTLSA
VlhA.5.08       GANKIEFSVPAEKVAPMIGNMYLTSSADEANKQKIENSIFGNSVTTENNRTIISVDALSG
                * *.****.* .**********:***  *:***:*************** * ****:**.
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                                      510                               540
                                       |                                 |
9.3                 YSLASDWSTFIGQYSSDSLTLNGNRMSDQKYYLIGYVGGNTGQRDIT-MVANTNQQRLPT
vlhA.3.07           YSLASDWSTFIGQYSSDSLTLNGNRISDQKYYLIGYVGGNTGQRNIT-MVANTNQQRLPT
VlhA.5.08           YSLASDWSTFVGRYTDQQLTLDGKMMPDQKYYLIGYVGGNTGARNVTGITEKTNEQRSPV
                    **********:*:*:.:.***:*: :.*************** *::* :. :**:** *.

                                      570                               600
                                       |                                 |
9.3                 ASNQNTRSYTLYVNAPKAGAYYIKGVFASEVRRDLKFSTGDMSSNN-VTIRQLSTGNLTT
vlhA.3.07           ASNQNTRSYTFYVNAPKAGAYYIKGVFTSEVRRDLKFSTGDMSSNN-VTIQQLTTGNLTT
VlhA.5.08           ASTPKTRTYTFYVNAPKAGAYYIKGVFASKVRRDLKFSTGDMSSNNNVTVRQLSTNNLTT
                    **. :**:**:****************:*:*************** **::**:*.****

                                      630                               670
                                       |                                 |
9.3                 LKTFDTSAITGPTQVTTVDTNRKTLTLVEGLNKIVVSGATADNGNAPNFGYLEFILNETQ
vlhA.3.07           LKTFDTSATEGPTRVTTVDTNRKTLTLVKGLNKIVVSGATANNGNAPNFGYLEFILNETQ
VlhA.5.08           LKTFDTSATTGPTRVTTVPDNRKTLTLVEGLNKIVVSGATADMGNAPNFGYLEFILNETQ
                    ********  ***:****  ********:************: ****************

                           680
                            |
9.3                 PETT------
vlhA.3.07           S---------
VlhA.5.08           PETTNVSNPS
                    .
```

Fig. 2: Alignment of the 9.3 orf, vlhA.3.07, and vlhA.5.08-predicted amino acid sequences. The vlhA.3.07 and vlhA.5.08 proteins are from *M. gallisepticum* R-strain (GenBank accession numbers NP_853377 and NP_853377) and the 9.3 orf is from *M. gallisepticum* F-strain (GenBank accession number AF275312). Identical amino acids are designated by an "*". Conservative amino acid substitutions are designated by "." and ":". Dashes indicate gaps introduced to maximize homology. The peptide sequences shown in Table 1 are underlined.

(Markham *et al.*, 1992). The molecular mass range for pMGA lipoproteins identified by Markham *et al.* (1992) is similar to the 70-kDa molecular mass of the F-strain protein recognized by mAb 6F10 (Garcia and Kleven, 1994; Wan *et al.*, 2004). We found that the majority of the peptides derived from the 70-kDa protein recognized by mAb 6F10 are homologous to vlhA proteins (Table 1 and Fig. 2), suggesting that a vlhA gene encodes the epitope recognized by mAb 6F10.

The turboSEQUEST database search revealed peptides that would be expected from a tryptic digest of a combination of the 9.3-orf predicted protein from the F-strain and the vlhA.3.07 predicted protein from the R-strain. Given that the complete vlhA gene family of the F-strain (32 genes) has yet to be fully characterized (Baseggio *et al.*, 1996), it is possible that a vlhA gene identical to the vlhA.3.07 gene of the R-strain is present in the F-strain genome. In light of this possibility, one interpretation of our results is that both the vlhA.3.07 and 9.3-orf genes are co-expressed in the F-strain and that both protein products share the epitope recognized by mAb 6F10. However, in a survey of different *M. gallisepticum* strains, it was found that at the protein level, the S6 strain expressed the pMGA1.1 gene, while the R strain expressed the pMGA1.2 gene. In RT-PCR experiments, low levels of mRNA for two additional pMGA genes (pMGA1.4 and pMGA1.8) were also found in the S6 strain population. However, only the pMGA1.1 gene expression was detected at the protein level (Glew *et al.*, 1995). Therefore, based on the expression studies conducted to date (Markham *et al.*, 1998; Glew *et al.*, 1998; Berlic *et al.*, 2000), a more likely explanation would be that mAb 6F10 recognizes the product of a previously uncharacterized vlhA gene that is highly similar to both the 9.3-orf and vlhA.3.07 genes.

In studies examining vlhA protein expression in the F-strain (Glew *et al.*, 1995), the 75-kDa protein identified with vlhA-specific mAb by Markham *et al.* (1992) was characterized by amino acid sequencing. The N-terminal 18 amino acids of the protein that could be clearly deduced showed high similarity to the N-terminus of the pMGA1.2 protein. However, in previous studies from our laboratory examining the expression of pMGA gene mRNA in the F-strain, only the 9.3-orf cDNA was

Table 1: Mass spectrometry analysis of tryptic peptides derived from the 70-kDa protein recognized by mAb 6F10.

Peptide sequence	Alignment with VlhA proteins	Amino acid position
VlhA-specific peptides (*M. gallisepticum*)		
TALTSLLASK (5) a	9.3 orf/vlhA.3.07, 5.08	85 - 94 b
NANIEMYSDYAK (2)	9.3 orf	95 - 106
NATSALQTAINTANSNK (12)	9.3 orf/vlhA.3.07	134 - 150
NATSALQTAINTANSNKQK (12)	9.3 orf/vlhA.3.07	134 - 152
QKFDQDHSNLLMSYK (9)	9.3 orf/vlhA.3.07	151 - 165
FDQDHSNLLMSYK (9)	9.3 orf/vlhA.3.07	153 - 165
KETTVMTLKDPK (4)	9.3 orf	174 - 185
YSAILDQINGVSSK (8)	vlhA.3.07	186 - 199
NNADQFANYQSFTLDK (2)	9.3 orf/vlhA.3.07	242 - 257
AIFTSGNQPTK (3)	9.3 orf/vlhA.3.07	306 - 316
ANDDVGLQYK (30)	9.3 orf/vlhA.3.07	371 - 380
VRLTGLAFGK (12)	9.3 orf/vlhA.3.07	413 - 422
LTGLAFGK (3)	9.3 orf/vlhA.3.07	415 - 422
YYLIGYVGGNTGQR (2)	9.3 orf/vlhA.3.07	511 - 524
NITMVANTNQQR (8)	vlhA.3.07	525 - 534
DLKFSTGDMSSNNVTIR (6)	9.3 orf	574 - 591
FSTGDMSSNNVTIR (6)	9.3 orf/vlhA.3.07	577 - 591
VTTVDTNRKTLTLVK (4)	vlhA.3.07	615 - 629
KTLTLVK (2)	vlhA.3.07	623 - 629
Conserved hypothetical (*M. gallisepticum*)		
EVTGEGNSITPNADPQKAK	-c	-
ABC Transporter ATP-binding (*M. pulmonis*)		
EIQLQNLKIQFDK	-	-

a Number of times the peptide was identified. b Location of the peptide sequence within the vlhA proteins aligned in Fig. 2.
c The peptides from the *M. gallisepticum* conserved hypothetical protein and the peptide from the *M. pulmonis* ABC transporter do not show homology with any vlhA protein in the database.

reproducibly detected (Pharr *et al.*, 2002). In amino acid sequence alignments, the 9.3-orf showed 96% amino acid identity with the predicted product of the vlhA.3.07 gene, but only 60% identity with the pMGA1.2 protein. Expression of different vlhA genes in different isolates of the F-strain is conceivable. Phase variation in vlhA gene expression has been suggested as a mechanism used by *M. gallisepticum* to avoid host immune responses (Glew *et al.*, 2000) and antibody driven selection has been shown to induce a shift in vlhA gene expression (Markham *et al.*, 1998; Glew *et al.*, 1998). Therefore, it is plausible that different isolates of the F-stain vaccine could express different vlhA genes, possibly explaining our identification of products of the 9.3-orf or vlhA.3.07 genes in the F-strain isolate used in our studies, rather than the pMGA1.2 gene product found by Glew *et al.* (1995).

Previous studies of phase and size variation demonstrated by the protein recognized by mAb 6F10 in different F-strain isolates derived from experimentally infected chickens (Garcia and Kleven, 1994) may in part be explained by the findings of Berlic *et al.* (2000). They conducted a large study in which pMGA gene expression was examined in isolates of virulent *M. gallisepticum* obtained from chickens throughout Europe and the United States. Like previous studies, they showed that *M. gallisepticum* expressed only a limited repertoire of the pMGA genes encoded in the genome. Isolates expressed either the pMGA1.2 or pMGA1.9 gene, but not both. However, in those isolates that expressed the pMGA1.2 gene, analysis of the primary sequence revealed the duplication or deletion of short stretches of nucleotide sequence. Moreover, sequence modifications within the pMGA1.2 gene in some isolates resulted in the loss of epitopes recognized with a panel of pMGA-specific mAb. The potential for sequence modification of vlhA genes may therefore account for the molecular weight variability observed in the expression of the F-strain vlhA protein detected with mAb 6F10 (Garcia and Kleven, 1994). In theory, the kind of genetic variability described by Berlic *et al.* (2000) could create a vlhA open reading frame representing a composite of the vlhA.3.07 and 9.3-orf sequences. Future studies will be required to address this possibility.

In summary, our results suggest that a vlhA gene product encodes the epitope recognized by mAb 6F10. Analysis of the 70-kDa protein reactive with mAb 6F10 revealed peptides predicted from tryptic digests of the 9.3-orf and vlhA.3.07 gene products. Therefore, two highly similar vlhA genes may share the 6F10-specific epitope, or alternatively a product of a vlhA gene representing a composite of the vlhA.3.07 and 9.3-orf sequences could encode the epitope. At present, our data do not distinguish between these possibilities, and further studies will be required to identify the vlhA gene(s) that express the product recognized by mAb 6F10. This basic information will be important for future investigations to determine the role of the vlhA gene family in the efficacy of the F-strain vaccine.

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