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Author(s): Aaron M. Lynne, Steven L. Foley, Lisa K. Nolan
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Research Note—

Characterization of Monoclonal Antibodies to Avian *Escherichia coli* Iss

Aaron M. Lynne,[A] Steven L. Foley,[B] and Lisa K. Nolan[AC]

[A]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50010
[B]Marshfield Clinic Research Foundation, Marshfield, WI 54449

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SUMMARY. Colibacillosis accounts for annual multimillion dollar losses in the poultry industry, and control of this disease is hampered by limited understanding of the virulence mechanisms used by avian pathogenic *Escherichia coli* (APEC). Previous work in our laboratory has found that the presence of the increased serum survival gene (*iss*) is strongly associated with APEC but not commensal *E. coli*, making *iss* and the protein it encodes (Iss) candidate targets of colibacillosis-control procedures. Previously, we produced monoclonal antibodies (MAbs) against Iss to be used as a reagent in studies of APEC virulence and colibacillosis pathogenesis. Unfortunately, the utility of these MAbs was limited because these MAbs exhibited nonspecific binding. It was thought that the lack of specificity might be related to the fact that these MAbs were of the immunoglobulin M (IgM) isotype. In the present study, new MAbs were produced using a different immunization strategy in an effort to generate MAbs of a different isotype. Also, because Iss bears strong similarity to Bor, a lambda-derived protein that occurs commonly among *E. coli*, MAbs were assessed for their ability to distinguish Iss and Bor. For these studies, the *bor* gene from an APEC isolate was cloned into an expression vector. The fusion protein expressed from this construct was used to assess the potential of the anti-Iss MAbs produced in the past and present studies to distinguish Bor and Iss. The MAbs produced in this study were of the IgG_1 isotype, which appeared to bind more specifically to Iss than previously generated antibodies in certain immunologic procedures. These results suggested that the MAbs generated in this study might prove superior to the previous MAbs as a reagent for study of APEC. However, both MAbs recognized recombinant Iss and Bor, suggesting that any results obtained using anti-Iss MAbs would need to be interpreted with this cross-reactivity in mind.

RESUMEN. *Nota de Investigación*—Caracterización de anticuerpos monoclonales contra la proteína Iss de *Escherichia coli* aviar. La colibacilosis es responsable anualmente por pérdidas multimillonarias en la industria avícola y el control de esta enfermedad está restringido por el limitado conocimiento de los mecanismos de virulencia utilizados por las cepas patógenas aviares de *Escherichia coli*. Trabajos previos en nuestro laboratorio han encontrado que la presencia del gen de supervivencia incrementada en suero (iss por sus siglas en Inglés) está fuertemente asociada con las cepas patógenas aviares de *E. coli*, pero no con las cepas normales o no patógenas de *E. coli*, lo que hace del gen de supervivencia incrementada en suero y la proteína para la cual codifica (por sus siglas en inglés Iss) el blanco de los procedimientos de control para colibacilosis. Con anterioridad, produjimos anticuerpos monoclonales contra la proteína Iss para ser utilizados en los estudios de virulencia de las cepas patógenas aviares de *E.coli* y la patogénesis de la colibacilosis. Desafortunadamente, la utilidad de estos anticuerpos monoclonales fue limitada debido a que los anticuerpos mostraron uniones inespecíficas. Se pensó que la falta de especificidad podría estar relacionada con el hecho de que estos anticuerpos monoclonales eran del isotipo IgM. En el presente estudio, se produjeron nuevos anticuerpos monoclonales utilizando una estrategia de inmunización diferente, tratando de generar anticuerpos monoclonales de un isotipo distinto. Así mismo, debido a que la proteína Iss posee una fuerte similitud con una proteína derivada–Lamda (Bor) de ocurrencia común entre las cepas de *E. coli*, los anticuerpos monoclonales se evaluaron por su capacidad para distinguir entre Iss y Bor. Para estos estudios el gen *Bor* de un aislamiento de *E. coli* patógena aviar se clonó en un vector de expresión. La proteína de fusión expresada de este ensamble se utilizó para evaluar el potencial de los anticuerpos monoclonales anti- Iss producidos en los estudios anteriores y en los presentes de distinguir entre Iss y Bor. Los anticuerpos monoclonales producidos en este estudio fueron del isotipo IgG_1 los cuales en ciertos procedimientos inmunológicos se unieron más específicamente a la proteína Iss que los anticuerpos generados con anterioridad. Estos resultados sugieren que los anticuerpos monoclonales generados en este estudio pueden ser superiores a los anticuerpos monoclonales previos, como un reactivo para el estudio de las cepas patógenas aviares de *E. coli*. Sin embargo, ambos anticuerpos monoclonales reconocieron la proteínas Iss y Bor recombinantes, lo que sugiere que cualquier resultado obtenido utilizando anticuerpos monoclonales Iss tendría que ser interpretado teniendo en cuenta esta reactividad cruzada.

Key words: APEC, avian colibacillosis, Bor, Iss, monoclonal antibody

Abbreviations: APEC = avian pathogenic *Escherichia coli*; Bor = blue open-reading frame protein; DMEM = Dulbecco's modified Eagle medium; ELISA = enzyme-linked immunosorbent assay; GST = glutathione-*S*-transferase; Ig = immunoglobulin; Iss = increased serum-survival protein; IPTG = isopropyl-β-D-thiogalactopyranoside; LB = Luria–Bertani; MAbs = monoclonal antibodies; PCR = polymerase chain reaction; PEG = polyethylene glycol; PVDF = polyvinylidine difluoride; SDS-PAGE = sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tm = melting temperature

Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is a major problem for the poultry industry in the United States, resulting in annual multimillion-dollar losses (1). One of the problems encountered in colibacillosis control is that no single bacterial trait has been identified that can be used as an identifier of APEC strains (1). Previous work has shown that complement resistance may play an important role in APEC virulence (11,13,15,22), suggesting that traits contributing to complement resistance might have value as identifiers of APEC strains. Previously, it was shown that the increased serum survival (*iss*) gene (4), which is associated with *E. coli* complement resistance (4,5,6), was found significantly more often in APEC than it was in commensal *E. coli*

[C]Corresponding author.

isolates of apparently healthy birds (16,18). This strong association between *iss* and disease-causing *E. coli* of birds suggested to us that *iss*-centric strategies might be useful in controlling colibacillosis and in studying the pathogenesis of avian colibacillosis.

The *iss* gene was first described by Binns *et al.* (4) for its role in complement resistance associated with a ColV plasmid of a human *E. coli* isolate. Iss, the protein product of *iss*, is thought to occur as a 10–11-kDa lipoprotein in the bacterial outer membrane (3,10). *iss* may be a derivative of *bor*, a gene of bacteriophage λ (2,3,5,10). The blue open-reading (Bor) frame protein, a lipoprotein of the cell envelope of *E. coli* λ lysogens, appears to confer complement resistance on these lysogens (2). Amino acid sequences of Iss and Bor are about 90% identical (10).

Previously, we produced monoclonal antibodies (MAbs) against recombinant Iss to be used as a reagent in the study of APEC virulence and control (7). Using a ''two fusion protein approach,'' where two different Iss fusion proteins were used to immunize mice, MAbs were generated that recognized recombinant Iss. Isotyping of these MAbs revealed that they were of the immunoglobulin (Ig) M isotype. It was felt that IgM MAbs might be less desirable for diagnostic purposes than those of the IgG type because IgM antibodies are larger and bind less specifically than IgG antibodies (19).

Further, IgG antibodies may be preferred for number of reasons. First, they are easier to purify from tissue-culture supernatants, allowing for more concentrated antibody preparations for antibody-testing regimes (9). Second, IgG antibodies have a longer half-life than IgM, which may be important for maintaining the stability and affinity of antibodies for use in diagnostics (19). Finally, IgG molecules have more flexibility, allowing them to better align with the antigenic portion of a molecule, providing a higher affinity to antigens than IgM (9). This trait is important in increasing the specificity of diagnostic methods. Additionally, a factor determining the utility of anti-Iss MAbs could lie in their ability to discriminate between Iss and Bor because Bor is quite similar in structure to Iss and is widespread among *E. coli* (10).

Therefore, a MAb of the IgG isotype, able to discriminate between Iss and Bor proteins, was sought in this study. It is thought that such a MAb could prove useful as a reagent for the study of avian *E. coli* and avian colibacillosis.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains used were *E. coli* BL21 (DE3) (Stratagene, La Jolla, CA) and *E. coli* JM109 (Promega, Madison, WI). *E. coli* BL21 was used for expression of GST–Iss and GST–Bor and *E. coli* JM109 was used in the creation of the pALbor construct. All strains were maintained on Luria-Bertani (LB) agar (Difco, Detroit, MI) with ampicillin (100 μg/ml; Amresco, Solon, OH) where appropriate. The plasmids used in this study were pGEM-T Easy (Promega), pGEX-6P-3 (Pharmacia Biotech, Piscataway, NJ), pLN320, pLN330, and pALbor. pGEM-T Easy is a TA cloning vector designed for easy cloning of polymerase chain reaction (PCR) products. pGEX-6p-3 is a plasmid-expression vector designed for production of proteins fused to glutathione-*S*-transferase (GST). pLN330 is composed of pGEX-6P-3 with the *iss* gene sequence (GenBank accession AF0422279) without the coding region for the signal sequence (the first 72 nucleotides of *iss*) (8). pLN320 is composed of pGEX-6P-3 with the *iss* gene sequence with the coding region for the signal sequence (8). pALbor is composed of pGEX-6P-3 with the *bor* gene sequence (GenBank accession X55792).

Molecular cloning. To amplify the *bor* gene for creation of pALbor, the upper primer was 5′ TTTTCTACACATACGATTCT-GCGAACT 3′, melting temperature (Tm): 56.5 C, and lower primer 5′ CTCGATGCAAAATACACGAAGGAGTTAGCT 3′, Tm: 60.1 C. The amplification program used was 94 C for 5 min; 30 cycles of 94 C for 30 sec, 55 C for 30 sec, 72 C for 30 sec; then 72 C for 7 min, and hold at 4 C. The amplified *bor* gene was prepared for ligation using the Wizard PCR preps DNA purification system (Promega). The *bor* amplicon was ligated into pGEM-T Easy, and the recombinant plasmid DNA was used to transform *E. coli* JM109 competent cells according to manufacturer's protocol (Promega). Plasmid DNA was purified from these transformants using Wizard *Plus* Minipreps DNA Purification System (Promega) and digested with *Eco*RI for 2 hr at 37 C. Digests were run on 1% low-melt agarose gel, stained with ethidium bromide, and viewed under ultraviolet light. A 648-base-pair band, corresponding in size to the predicted *bor* amplicon, was excised from the gel and purified from the agarose using MinElute Gel Extraction Kit (Qiagen, Valencia, CA). *Eco*RI-digested *bor* was ligated into pGEX-6P-3 according to manufacturer's protocol (Pharmacia) to form pALbor. Ligations were transformed in BL21 (DE3) using the calcium chloride method (20).

DNA sequencing. Clones were sequenced to confirm inserts were in the correct orientation and were ''in frame'' for protein expression. Double-stranded plasmid clones were sequenced at the Iowa State University DNA Sequencing and Synthesis Facility (Ames, IA) with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA).

Protein expression. The GST–Iss and GST–Bor fusion proteins were generated as described below. Briefly, *E. coli* BL21 (DE3) containing either pLN330 or pALbor were grown overnight on LB plates supplemented with ampicillin (50 μg/ml). Individual colonies were transferred to LB broth containing ampicillin (50 μg/ml), and the cultures were grown at 37 C with shaking to an optical density$_{600}$ of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the final concentration of 0.1 mM, and the cultures were incubated for an additional 4 hr. Protein expression was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Samples were loaded into wells of 15% Criterion gels (Bio-Rad) for separation in the Criterion System (Bio-Rad). Samples were electrophoresed at 200 V for 70 min. The gels were stained using SafetyBlue SafeStain (Invitrogen). Gels not stained were transferred to polyvinylidine difluoride (PVDF) membrane and probed with the anti-GST primary antibody and alkaline phosphatase–conjugated goat anti-mouse IgG secondary antibody. The membrane was developed using Bio-Rad immuno-blot color development reagent (5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolamine in dimethylformamide).

Protein purification. Lysis and solubilization of the GST–Iss fusion protein was accomplished following expression of pLN330. Induced cells were lysed with Bacterial-Protein Extraction Reagent (Pierce, Rockford, IL), and the inclusion body was solubilized with Inclusion Body Solubilization Reagent (Pierce) following manufacturer's protocol. GST–Iss was purified using an Immobilized Glutathione column (Pierce). For Iss purification, GST–Iss was cleaved using PreScission protease (Pharmacia), and cleaved GST was removed by passage through the Immobilized Glutathione column.

Hybridoma generation. Hybridoma generation was done in conjunction with Iowa State University's Hybridoma Facility (Ames, IA) following an approved Institutional Animal Care and Use Committee protocol. Briefly, three BALB/c mice were injected with 25 μg of GST–Iss in Freund's Complete Adjuvant subcutaneously. The mice were given two booster injections 2 wk apart with 25 μg of GST–Iss in Incomplete Freund's Adjuvant given intraperitoneally. Two weeks after the last boost, mice were bled, and the sera were tested to determine antibody titer using enzyme-linked immunosorbent assay (ELISA). Hybridomas were produced by the fusion of splenocytes from hyperimmune BALB/c mice and SP2/O mouse myeloma cells with the use of polyethylene glycol (PEG). The BALB/c mouse with the highest titer was euthanized by carbon dioxide asphyxiation, its spleen was aseptically harvested, and its splenocytes were collected by syringe perfusion with sterile Dulbecco's modified Eagle medium (DMEM) (13). Splenocytes and SP2/O cells were combined at a 1:1 ratio and centrifuged at 200 × *g* for 8 min. One milliliter of PEG was slowly added to the pellet, followed by the gradual addition of 10 ml of DMEM and incubation for 5 min at 37 C. The mixture was centrifuged at 200 × *g* for 8 min and

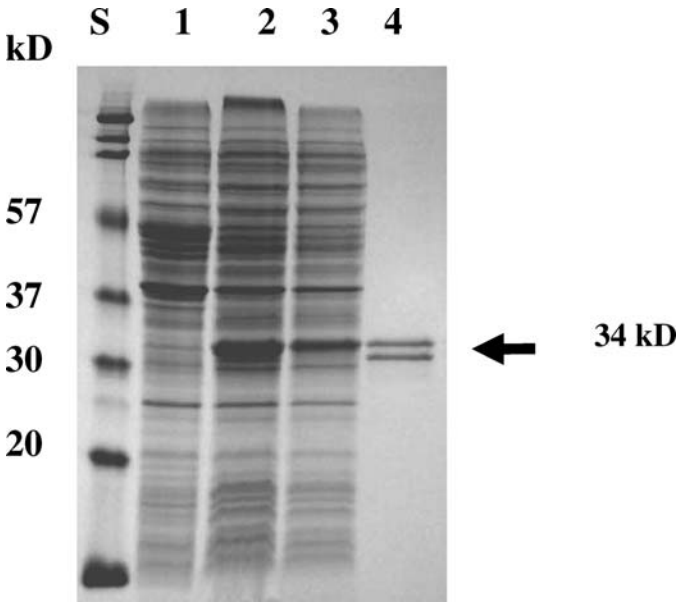

Fig. 1. SDS-PAGE of purified protein (GST–Iss) obtained after expression of pLN330 in BL21, lysis with Bacterial Protein Extraction Reagent, solubilization with Inclusion Body Solubilization Reagent, and purification with glutathione sepharose 4B. Lane S = the molecular weight standard (Bio-Rad prestained molecular weight standard) with sizes given in kilodaltons (kDs); lane 1 = BL21 cell lysate; lane 2 = cell lysate of pLN330 in BL21 induced with IPTG; lane 3 = column flow-through of BL21 pLN330 cell lysate; and lane 4 purified GST–Iss.

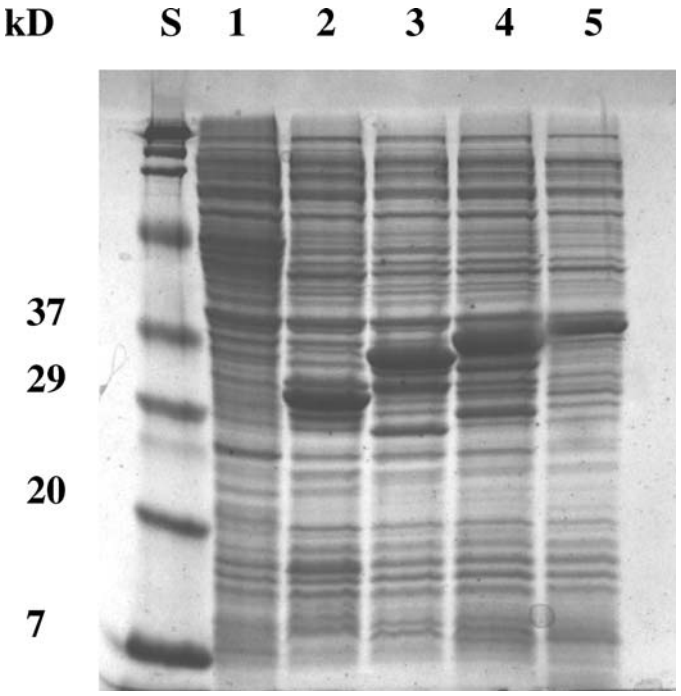

Fig. 2. SDS-PAGE to detect the presence of expressed recombinant proteins in BL21. Lane S = the molecular weight standard (Bio-Rad prestained molecular weight standard); lane 1 = BL21 cell lysate; lane 2 = BL21 with pGEX-6P-3 following induction with IPTG; lane 3 = BL21 with pLN330 following induction with IPTG; lane 4 = BL21 with pLN320 following induction with IPTG; and BL21 with pALbor following induction with IPTG. Recombinant proteins GST, GST–Iss without signal sequence, GST–Iss with signal sequence, and GST–Bor were expressed as shown in lanes 2–5, respectively.

resuspended in a volume of media containing DMEM, 10% horse serum, 0.1 mM hypoxanthine, 0.004 mM aminopterin, 0.016 mM thymidine, penicillin [63 μg/ml], streptomycin [0.1 mg/ml], fungizone [0.25 μg/ml], 2 mM L-glutamine, and 50% myeloma-conditioned media, such that the concentration was 2×10^6 cells/ml, and 0.2 ml of the hybridoma mixture was dispensed into a 96-well tray and incubated at 37 C. After 5 days, cells were fed with a mixture containing DMEM, 10% horse serum, 50% conditioned media, hypoxanthine and thymidine (without aminopterin) to select for hybridomas.

Hybridoma screening for anti-Iss antibody production. As soon as hybridoma growth was observed, cell-free supernatant fluid was screened for the presence of antibodies against Iss. Screening was done, as previously described (17) by ELISA, using cleaved Iss as the coating antigen.

Dilution cloning and expansion. Hybridomas that screened positive against Iss by ELISA were serially diluted in 96-well trays to isolate single clones (13). Wells with growth of a single colony were screened for Iss recognition by ELISA. Wells with MAbs that recognized Iss were sequentially expanded into flasks. During expansion, the amount of horse serum was reduced to 2%. Cell-free supernatant was collected for MAb purification (9,13).

Isotyping. After expansion, MAbs were characterized with Mouse Typer Sub-isotyping Kit (Bio-Rad) according to the manufacturer's protocol. Cleaved Iss was used as the coating antigen for the isotyping ELISA.

MAb purification. MAbs were purified using MAbTrap Kit (Amersham Biosciences) according to manufacturer's instructions.

Specificity of MAbs. To test the specificity of anti-Iss MAbs, lysates of *E. coli* BL21 (DE3), BL21 (DE3) containing pGEX-6P-3, BL21 (DE3) containing pLN320, BL21 (DE3) containing pLN330, and BL21 (DE3) containing pALbor were subjected to SDS-PAGE, blotted onto PVDF membranes and probed with anti-Iss MAbs (diluted 1:1000 in phosphate-buffered saline) as previously described (14).

RESULTS

To generate MAbs specific for Iss, a GST–Iss fusion protein was produced. Expression of GST–Iss from BL21 (DE3) containing pLN330 (pGEX-6P-3 containing *iss* without the signal sequence) was induced with IPTG. Cells were then harvested and lysed. Inclusion bodies were solubilized, and the fusion protein was purified by affinity chromatography (Fig. 1) and used for immunization. Recombinant Iss was produced for use in screening of MAbs (12). Iss was cleaved from its fusion partner with PreScission Protease, and its fusion partner was removed (12).

In an effort to help characterize anti-Iss MAbs, a recombinant Bor fusion protein was produced. To produce GST–Bor, *bor* was cloned into pGEX-6P-3. The resulting construct was termed pALbor. DNA sequencing of the construct confirmed that *bor* was ligated in the correct reading frame for proper expression. Cell lysates of induced BL21-(DE3) pALbor contained a 37-kD protein corresponding to the GST–Bor protein product (Fig. 2).

Mice immunized with GST–Iss were able to generate an immune response against Iss, as shown by ELISA, with sera from three immunized mice after their second boost of GST–Iss. Sera from immunized mice reacted with purified Iss protein at a dilution of 1:1000. Sera from unimmunized mice did not react with Iss. The mouse with the greatest antibody response to Iss was used to generate MAbs.

Two weeks after the fusion, hybridomas were screened for anti-Iss MAb production by ELISA. Ten wells gave a positive response and were subsequently subcloned. When growth was observed in the

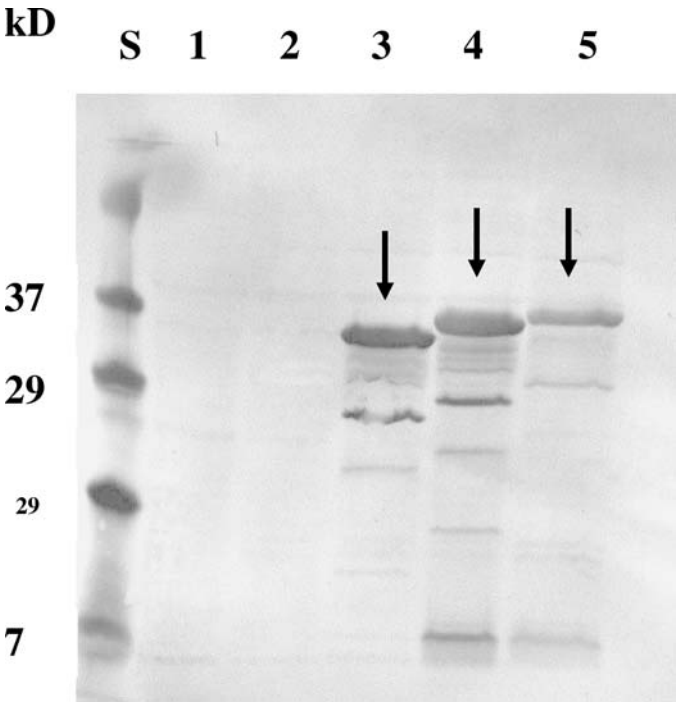

Fig. 3. Western blot to confirm the specificity of MAb 8B10-5B7. Lane S = the molecular weight standard (Bio-Rad prestained molecular weight standard); lane 1 = crude lysate of BL21 (DE3); lane 2 = crude lysate of BL21 with pGEX-6P-3 (expressing GST protein); lane 3 = crude lysate of BL21 with pLN330 (expressing GST–Iss without the signal sequence); lane 4 = crude lysate of BL21 with pLN320 (expressing GST–Iss with the signal sequence); and lane 5 = crude lysate of BL21 with pALbor. Arrows point to where MAbs bound to recombinant proteins of GST–Iss, with and without the signal sequence, and GST–Bor. It is thought that the bands seen below major bands, marked by the arrows, are breakdown products of the fusion proteins.

cloning plates, 40 subclones were rescreened by ELISA. Of the 40 subclones, the five that gave the strongest response were expanded and isotyped. Each subclone produced antibodies of the IgG_1 subclass with κ-light chains.

MAbs were evaluated for their specificity to Iss by western blotting. Of the five MAbs produced, MAb 8B10-5B7 showed the greatest affinity for Iss and was used for the remainder of the study. MAb 8B10-5B7 recognized bands corresponding in size to GST–Iss with and without the signal sequence. The antibody did not recognize any proteins from BL21 (DE3) alone or BL21 (DE3) with p-GEX-6P-3 (expresses GST only). MAb 8B10-5B7 also recognized a band corresponding to GST–Bor (Figs. 2, 3).

DISCUSSION

Avian colibacillosis is a costly disease to the poultry industry, accounting for multimillion-dollar losses annually (1). Previous work in our lab has shown that *iss* is strongly associated with APEC but not with fecal isolates from apparently healthy birds (16,18), making *iss* and the protein it encodes, Iss, potential identifiers of virulent isolates. To generate MAbs for use as reagents in the study of APEC and colibacillosis, *iss* was previously cloned into two different expression vectors to express two fusion proteins, GST–Iss and calmodulin-binding protein (CBP)–Iss (7). These proteins were used in a ''two-fusion protein immunization strategy'' to produce MAbs against Iss. The reasoning behind this approach was to select plasma cells producing antibodies specific for Iss by immunizing mice with GST–Iss for the first few immunizations, then switching to CBP–Iss for the last booster. Results of this strategy yielded MAbs of the IgM class. During subsequent characterization of these MAbs, it was found that they may bind nonspecifically to the surface of *iss*-negative controls, making use of these MAbs for diagnostic purposes problematic. It was thought that the lack of specificity of these MAbs might be due to their being of the IgM class and that the ''two-immunogen'' approach used in MAb generation may have skewed the immune response towards IgM production.

Consequently, in the present study, a single fusion protein immunization strategy, where mice were immunized with GST–Iss only, was used in antibody generation in an effort to generate MAbs of a non-IgM type. Using this approach, MAbs of the IgG type were successfully produced. However, production of anti-Iss MAbs using only GST–Iss presents other problems. For instance, GST is approximately three times larger than Iss, presenting the possibility that the majority of antibodies produced will be directed against GST not Iss. To detect hybridomas producing antibodies against Iss only, ELISA was performed with purified Iss cleaved from GST–Iss. During characterization of MAb 8B10-5B7, western blots were performed to see whether 8B10-5B7 would bind to GST without Iss. Results showed that MAb 8B10-5B7 would bind to GST–Iss with and without the signal sequence but not to GST alone, suggesting that the MAbs were directed against Iss alone.

Also of concern was the potential that these anti-Iss MAbs might cross-react with Bor, a protein showing strong homology to Iss (10). For these studies, a second fusion protein, GST–Bor was produced. MAbs were screened by western blot for their specificity to the Bor fusion protein. Results showed that MAb 8B10-5B7 did bind to recombinant Bor. Although these results are undesirable, they are not surprising given that Bor and Iss share about 90% homology (10). Further characterization will be necessary to determine whether such cross-reacting MAbs will be useful as reagents in the study to colibacillosis.

MAbs directed against outer-membrane proteins have been useful in the study and diagnosis of other organisms. For example, MAbs directed against outer-membrane proteins have been used in a surface-adhesion immunofluorescent technique for rapid detection of *Yersinia enterocolitica* in meat (21). MAbs could also be used to confirm the presence of Iss on the outer membrane and determine the level of Iss expression between virulent and avirulent isolates.

In spite of colibacillosis' devastating effects on the poultry industry, understanding of the virulence mechanisms of APEC have been hampered by the lack of a distinguishing characteristic of virulent isolates. From the results of Pfaff-McDonough *et al.* (16), *iss* appears to be a characteristic that can distinguish virulent from avirulent APEC isolates. In the present study, IgG MAbs were generated that may be a useful tool to confirm Iss's location on the bacterium, to study the interaction of Iss and its complement, and to detect virulent strains in the environment.

REFERENCES

1. Barnes, H. J. Colibacillosis in poultry. Pfizer, Inc., Lee's Summit, MO. 1994

2. Barondess, J. J., and J. Beckwith. A bacterial virulence determinant encoded by lysogenic coliphage λ. Nature 346:871–874. 1990.

3. Barondess, J. J., and J. Beckwith. *bor* gene of phage λ, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. J. Bacteriol. 177:1247–1253. 1995.

4. Binns, M. M., D. L. Davies, and K. G. Hardy. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. Nature 279:778–781. 1979.

5. Chuba, P. J., M. A. Leon, A. Banerjee, and S. Palchaudhuri. Cloning and DNA sequence of plasmid determinant Iss, coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Mol. Gen. Genet. 216:287–292. 1989.

6. Chuba, P. J., S. Palchaudhuri, and M. A. Leon. Contributions of *traT* and *iss* genes to the serum resistance phenotype of plasmid ColV2-K94. FEMS Microbiol. Lett. 37:135–140. 1986.

7. Foley, S. L., S. M. Horne, C. W. Giddings, T. R. Gustad, E. D. Handegard, M. Robinson, and L. K. Nolan. Monoclonal antibodies to avian *Escherichia coli* Iss. Avian Dis. 47:79–86. 2003.

8. Foley, S. L., S. M. Horne, C. W. Giddings, M. Robinson, and L. K. Nolan. Iss from a virulent avian *Escherichia coli*. Avian Dis. 44:185–191. 2000.

9. Goding, J. W. Monoclonal antibodies: principles and practice: production and application of monoclonal antibodies in cell biology, biochemistry, an immunology, 2nd ed. Academic Press, London, England. 1986.

10. Horne, S. M., S. J. Pfaff-McDonough, C. W. Giddings, and L. K. Nolan. Cloning and sequencing of the iss gene from a virulent avian *Escherichia coli*. Avian Dis. 44:179–184. 2003.

11. Ike, K., K. Kawahara, H. Danbara, and K. Kum. Serum resistance and aerobactin iron uptake in avian *Escherichia coli* mediated by conjugative 100-megadalton plasmid. J. Vet. Med. Sci. 54:1091–1098. 1992.

12. Lynne, A. M., S. L. Foley, and L. K. Nolan. Immune response to recombinant *Escherichia coli* protein Iss in poultry. Avian Dis. 50:273–276. 2006.

13. Mishell, B. B. and S. M. Shiigi. Selected methods in cellular immunology. W. H. Freeman, San Francisco, CA, 1980.

14. Nolan, L. K., R. E. Wooley, and R. K. Cooper. Transposon mutagenesis used to study the role of complement resistance in the virulence of an avian *Escherichia coli* isolate. Avian Dis. 36:398–402. 1992.

15. Nolan, L. K., S. M. Horne, C. W. Giddings, S. L. Foley, T. J. Johnson, A. M. Lynne, and J. Skyberg. Resistance to serum complement, Iss, and virulence of avian *Escherichia coli*. Vet. Res. Commun. 27:101–110. 2003.

16. Pfaff-McDonough, S. J., S. M. Horne, C. W. Giddings, J. O. Ebert, C. Doetkott, M. H. Smith, and L. K. Nolan. Complement resistance-related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Dis. 44:23–33. 2000.

17. Robinson, M., T. R. Gustad, M. R. Erickson, J. L. Ferguson, and C. S. David. Non-specific binding of IgG1 to *Heligmosomoides polygyrus*: adult worm homogenate superantigen is a target for immunoglobulin-induced inhibition. Parasitology 19:469–474. 1997.

18. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan. Characterizing the APEC pathotype. Vet. Res. 36: 241–256. 2005.

19. Roitt, I., J. Brostoff, and D. Male. Immunology, 2nd ed. Mosby Publ., London, England. 1996.

20. Sambrook, J., E. F. Fritsch, and T. Maniatis. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, NY. 1989.

21. Sheridan, J. J., C. M. Logue, D. A. McDowell, I. S. Blair, T. Hegarty, and P. Toivanen. The use of a surface adhesion immunofluorescent (SAIF) method for the rapid detection of *Yersinia enterocolitica* serotype O:3 in meat. J. Appl. Microbiol. 85:737–745. 1998.

22. Vidotto, M. C., E. E. Muller, J. C. de Freitas, A. A. Alfieri, I. G. Guimaraes, and D. S. Santos. Virulence factors of avian *Escherichia coli*. Avian Dis. 34:531–538. 1990.

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