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Production of Monoclonal Antibody for the Detection of Meat and Bone Meal in Animal Feed

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For the detection of prohibited meat and bone meal (MBM) in animal feed, monoclonal antibodies (MAbs) were raised against heat-stable *h*-caldesmon purified from bovine intestinal smooth muscle. The obtained hybridoma cells were screened against extracts of the bovine MBM and heat-treated smooth muscle, and MAb 5E12 was identified as having the best performance. Antibody 5E12 did not react with animal feed, milk product, plant proteins, and other ingredients used for commercial animal feed except for the gelatin. This antibody diluted to 100-fold was able to detect MBM mixed in animal feed at 0.05% in an ELISA, and it showed strong affinity toward bovine smooth muscle autoclaved at 130 °C. Therefore, this antibody can be used in the ELISA system for field testing of the presence of MBM in animal feed.

KEYWORDS: Meat and bone meal; animal feed; monoclonal antibody; ELISA; immunoassay

INTRODUCTION

Transmissible spongiform encephalopathy (TSE) agents induce fatal neurodegenerative diseases in mammalian species and humans (*1*). The TSE group in animals includes scrapie in sheep and goat, bovine spongiform encephalopathy (BSE) in cattle, feline spongiform encephalopathy, transmissible mink encephalopathy, and chronic wasting disease in wild ruminants. Among them, BSE, commonly known as mad cow disease, has brought enormous economic consequences since its first incidence in the United Kingdom in 1986 (*2*). The BSE outbreaks peaked in the United Kingdom in 1993 at almost 1000 new cases per week, and it caused more than 182000 cases between 1988 and 2002 (*3*). In addition, the emergence of a new variant form of Creutzfeldt-Jakob Disease (vCJD) in humans in the United Kingdom has been proposed to be possibly linked with BSE (*4*, *5*).

Meat and bone meal (MBM), an ingredient of animal feed, contaminated with a TSE agent was believed to be the major vehicle of BSE transmission, according to epidemiological inquiry (*1*). MBM has been produced by rendering the discarded animal fat, bones, offal, and whole carcasses from bovine, ovine, porcine, and poultry. Although the use of MBM in cattle as a nitrogen supplement had been a common practice for several decades, changes in rendering operations in the 1970s and 1980s may have allowed the survival of the contagious agents that can be transmitted to the cattle through the MBM (*2*). The oral route was the major mode of natural transmission of BSE to cattle (*6*). To prevent the spread of BSE, the European Union in 1988 banned the inclusion of ruminant-derived proteins in animal feed (*2*). The U.S. Food and Drug Administration (FDA) also introduced the feed ban in 1997 to prohibit the use of proteins derived from mammalian tissues in feeding ruminants (21 CFR 589.2000) (*7*).

The U.S. Department of Agriculture (USDA) announced the first outbreak of BSE in December 2003. The BSE-positive Holstein cow found in Mabton, WA, originated from a dairy farm in Alberta, Canada (*8*). The USDA veterinarians believed that this diseased Holstein was born a few months before the United States and Canada banned the use of MBM in animal feed in 1997. The incident has revived public concern about the safety of consuming beef and other animal-derived products.

Despite the regulation on feed ban, MBM may still enter cattle diets accidentally as a result of cross-contamination during feed mixing at the feed mills, transportation, storage, or the farm (*3*). Indeed, the FDA found very low levels of prohibited MBM residues in the feedlot resulting from misformulation of the animal feed supplement at feed mills (*9*). Thus, identifying the tools that will permit enforcement of the bans implementation to eradicate BSE is critical for compliance with animal byproduct regulation (*10*). However, there are technical limitations in

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detecting prohibited MBM residues in animal feed because most of the analytical methods cannot distinguish between allowable and prohibited bovine materials (*11*). In addition, not many methods are available as quantitative assay systems for MBM analysis. Therefore, in this study, monoclonal antibodies (MAbs) were raised against a heat-stable biomarker and used to develop an immunological assay for the detection of MBM residues in animal feed.

MATERIALS AND METHODS

Preparation of Sample Extracts. Bovine intestine was collected from a processor immediately after slaughtering. The collected samples were placed in ice-chilled water containing 0.25 mM phenylmethanesulfonyl fluoride (PMSF) and 0.75 mM benzamidine and transported in ice to the laboratory. The intestine samples were treated by removing the fat and inside mucus lining (*12*). The prepared samples were then frozen in liquid nitrogen, ground using a Waring blender, and stored at −80 °C. The sample powder (20 g) was homogenized in 120 mL of extraction buffer (300 mM KCl, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.25 mM PMSF, 0.01% NaN_3, 1 mM benzamidine, and 50 mM imidazole−HCl; pH 6.9) with a Polytron (Brinkmann Instruments, Westbury, NY). The crude extracts were centrifuged at 40000*g* for 30 min. For the heat treatment, the extracts were placed in a boiling water bath at 90 °C for 10 min and then centrifuged at 40000*g* for 30 min. The supernatants were collected as the heat-treated samples. To evaluate the effect of heat treatment on the integrity of the proteins, the supernatants were further autoclaved at 130 °C for 10, 30, 60, and 120 min.

Bovine and porcine MBM, animal feeds, and individual ingredients of the commercial animal feeds were collected from Cargill (Minneapolis, MN) and TBC (Birmingham, AL). To evaluate the sensitivity of the assay system, test samples were prepared by mixing different amounts of MBM (0.05, 0.1, 0.5, and 5%) with animal feed. Each sample (20 g) was homogenized in 60 mL of the extraction buffer and processed as described above. The protein concentrations of the extracts were determined by using the Bio-Rad protein assay (Bio-Rad Laboratory, Hercules, CA) with bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) as the standard.

Production of Monoclonal Antibody (Mab). Two different types of antigens were prepared for immunization in mice. Heat-stable protein, *h*-caldesmon, was purified from bovine intestine following the method of Lynch and Bretscher (*12*). Heat-treated smooth muscle extract as described above had $(NH_4)_2SO_4$ added to 30% saturation and centrifuged at 40000*g* for 20 min. The collected supernatant was added with $(NH_4)_2SO_4$ to 40% saturation and centrifuged at 40000*g* for 20 min. The precipitate was collected and dissolved in 20 mL of buffer (0.1 M NaCl, 0.1 M EGTA, 10 mM imidazole−Cl, 1 mM DTT, and 0.01% NaN_3; pH 7.0) and dialyzed in 1 L of the buffer (30 mM NaCl, 10 mM imidazole−Cl, 1 mM DTT, and 0.01% NaN_3; pH 7.0) overnight at 4 °C. Protein was applied to a 15 mL Whatman DE-52 anion-exchange column equilibrated with the buffer used for dialysis. Caldesmon was eluted with 50 mL of the buffer with a linear gradient of 0−250 mM NaCl.

Crude extract of MBM and purified MBM extract were also used for immunization. The crude extract of MBM that was prepared as described above was further purified using a dye affinity chromatography. Two milliliters of MBM extract was loaded onto a column packed with Reactive Yellow 86 (Sigma) or Reactive Blue 72 (Sigma). The column was washed with a 10-bed volume of washing buffer (10 mM Tris·Cl, 1 mM $CaCl_2$, 1 mM DTT, and 0.01% NaN_3; pH 7.5) and eluted with elution buffer (250 mM NaCl, 10 mM Tris·Cl, 1 mM $CaCl_2$, 1 mM DTT, and 0.01% NaN_3; pH 7.5). The collected fractions (0.8 mL each) were tested against MBM on ELISA and used for immunization.

MAb was produced at the Auburn University Hybridoma Facility according to the standard method of Kohler and Milstein (*13*) with modification. Three BALB/c mice were immunized with antigens (1 mg/mL) mixed with Freund's complete adjuvant (1:1, v/v). Subcutaneous injections of antigens (1.0 mg/mL) mixed with Freund's incomplete adjuvant were carried out at 3−4 week intervals until a strong immune response to the antigen was produced. To check the immune response, test sera from mice were periodically collected to determine the titer of the sera. For hybridoma production, the lymphocytes collected from the spleen of the immunized mouse were fused with Ag8 myeloma cells (ATCC CRL 1580) using a poly(ethylene glycol) fusion medium and then plated on 96-well plates in aminopterin−hypoxanthine−thymidine (HAT) medium with peritoneal macrophage feeder cells. The cells were cultured in a CO_2 incubator at 37 °C. The supernatants of cell cultures were screened for the presence of antibodies binding to heat-treated bovine smooth muscle extract and MBM using the enzyme-linked immunosorbent assay (ELISA). The hybridomas of interest were cloned by limiting dilution and extended. The isotypes of MAbs were determined by a mouse monoclonal antibody isotyping kit (Sigma) according to the manufacturer's instruction.

ELISA. ELISA was carried out to screen hybridoma cells capable of reacting with extracts of bovine smooth muscle and MBM and to determine the sensitivity of MAb 5E12 (*14*). Antigens (2 μg/well) were coated on the 96-well plate overnight at 4 °C and washed three times with washing buffer (0.1% Tween 20 in phosphate-buffered saline, PBS, pH 7.4, v/v). The plate was blocked with 1% BSA in PBS for 1 h. The test MAbs were added into the wells and incubated for 2 h, followed by binding with the secondary antibody, goat anti-mouse IgM or IgG alkaline phosphatase conjugate (1:3000, Sigma), for 1 h. The substrate, *p*-nitrophenyl phosphate (pNPP, Sigma), was added, and the color development was quantitatively measured at 405 nm using an ELISA reader (Bio-Rad Laboratory).

Analyses by SDS-PAGE and Western Blot. SDS-PAGE was carried out using a Mini-PROTEINII electrophoresis cell (Bio-Rad Laboratory) according to the method of Laemmli (*15*). Total proteins were separated on 10% of separating gel and 4% of stacking gel. Gels were stained with Coomassie Brilliant Blue G-250 (Bio-Rad Laboratory). For the Western blot, the separated proteins in the SDS-PAGE gel were electrotransferred into nitrocellulose membrane (Bio-Rad Laboratory) using a semidry blotter unit (Amersham Biosciences, San Francisco, CA) at 0.8 mA/cm^2 for 40 min using Towbin's buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, and 20% methanol) (*16*). The blot was blocked in 3% BSA for 1 h followed by 2 h of incubation with MAbs diluted to appropriate ranges. The binding of MAb onto the blot was detected by the secondary antibody enzyme conjugate, goat anti-mouse IgM peroxidase (1:500, Sigma). The blot was developed by adding 10 mL of Tris−Cl buffer (pH 7.0) containing 6 mg of 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 10 μL of 30% H_2O_2.

Confocal Microscopic Immunohistochemistry of Bovine Intestine Tissue. Autoclaved bovine intestine was immunolabeled with MAb 5E12 and other antibodies obtained in this study (*17*). Tissues were fixed overnight at 4 °C in 4% paraformaldehyde and 0.2 M Millonig's phosphate buffer. The fixed samples were rinsed in 20 mM PBS containing 0.1% NaN_3 and 0.1% Triton X-100. The fixed tissues were dried onto spot slides, and immunocytochemical reactions were performed at 5 °C with agitation on a shaker table. The fixed tissues were incubated in 30 μL of blocking medium consisting of 5% heat-inactivated goat serum in 20 mM PBS and, then, incubated overnight in test antibodies, the anti-acetylated tubulin antibody (Sigma), or a mouse monoclonal anti-small cardioactive peptide. All tissues were rinsed four times in PBS and followed by incubation overnight with a secondary antibody, donkey anti-mouse IgG or IgM conjugated to AlexaFluor 594 (1:200, Molecular Probes, Eugene, OR). Fluorescent images were captured using a Bio-Rad MRC-1000 laser scanning confocal microscope quipped with a krypton−argon laser.

Statistical Analysis. The sensitivity of MAb 5E12 in detecting MBM in animal feed was carried out in duplicate, and the test was replicated three times. The obtained data were analyzed by analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL). Significance of difference was defined at $p < 0.05$.

RESULTS

Screening of Hybridoma Cells. MBM and high molecular weight caldesmon (*h*-caldesmon) were used to raise antibodies

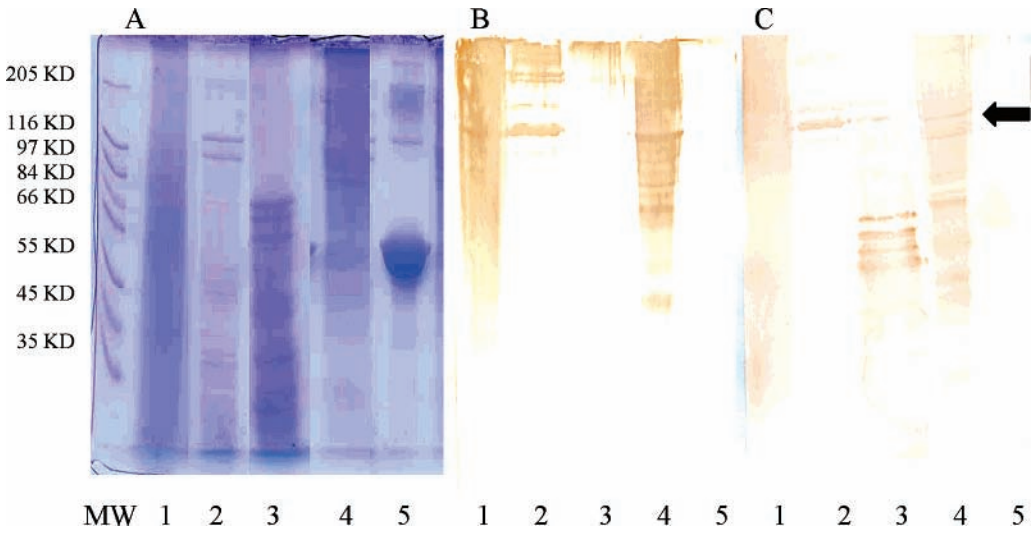

Figure 1. Western blot analysis using MAbs 5E12 and 2C8. Proteins separated by SDS-PAGE were stained for total proteins by (**A**) Coomassie blue R-250 and immunostained by (**B**) 5E12 (1:1000) or (**C**) 2C8 (1:1000): (lane 1) heat-treated MBM (6 μg); (lane 2) heat-treated bovine smooth muscle (10 μg); (lane 3) heat-treated animal feed (10 μg); (lane 4) gelatin (10 μg); (lane 5) BSA (10 μg).

for immunoassay development. A good immune response was observed in mice immunized with *h*-caldesmon. However, the extracts of the crude and purified MBM failed to induce good antibody responses. The serially diluted blood sera from the immunized mouse showed consistently low titration values against MBM. Therefore, *h*-caldesmon was considered to be a better antigen than MBM for immunization in mouse and MAb production.

Hybridoma cells raised against *h*-caldesmon were primarily screened against heat-treated MBM and bovine smooth muscle extracts by using ELISA and Western blot. Among the 800 supernatants of hybridoma cells tested, 155 samples showed reactivity with the two antigens tested (data not shown). Positive cells were further screened with different species (bovine, porcine, and chicken) of smooth muscle and MBM extracts, and their specific affinity onto the blot of *h*-caldesmon was confirmed by Western blot analysis (data not shown). The cells of interest were immediately cloned. The class of MAb obtained in this study was mostly IgM. Among the clones, MAbs 2C8 and 5E12 showed strong reactivity with MBM extracts (>3.0), but relatively low reactivity against bovine smooth muscle (<0.5) using ELISA. The remaining clones, such as 6F3, 7F3, 7G1, and 8G5, showed relatively low reactivity with the two antigens tested. Therefore, only MAbs 2C8 and 5E12 were further tested.

Cross-Reactivity of 5E12 against Ingredients Used in Commercial Animal Feed. The blots of *h*-caldesmon corresponding to ~150 kDa were detected in MBM and bovine intestine samples by immunostaining with MAb 5E12 and 2C8 (**Figure 1**). Although MAb 2C8 originally showed the strongest reactivity with extracts of heat-treated bovine smooth muscle and MBM on ELISA, it showed cross-reactivity with animal feed on Western blot assay. Therefore, MAb 2C8 was not tested further in this study. Only MAb 5E12 showed no reactivity with animal feed, BSA, and milk protein on Western blot and ELISA (**Figure 1** and **Table 1**).

MAb 5E12 was further tested for cross-reactivity with 15 different commercial ingredients used in animal feed. Three different dilutions (100-, 250-, and 500-fold) of this MAb were tested to optimize the assay system. The antibody showed strong affinity against both the bovine and porcine MBM, with the strongest ELISA signal observed with the bovine MBM. MAb 5E12 at 500-fold dilution showed a good activity ($OD_{405} > 1.5$) with bovine MBM, but a very strong signal ($OD_{405} > 3.0$) at 100-fold dilution (**Figure 2**). This antibody also reacted with porcine MBM, although its reactivity with the test sample was slightly lower than that of bovine MBM. The antibody did not react with most of the plant protein and other ingredients tested, such as wheat, high-fat rice bran, soy hulls, dried egg, corn, soybean meal, blood meal, dried porcine plasma, dried whey, and barley. However, MAb 5E12 showed cross-reactivity with gelatin and other gelatin-containing products, such as feather meal and fish meal.

Table 1. Sensitivity of the MAb 5E12 at 100- and 250-fold Dilutions in Detecting MBM Mixed in Animal Feed

ratio (%)		OD at 405 nm	
MBM	animal feed	1:100	1:250
100	0	2.577 ± 0.018	1.409 ± 0.057
5	95	0.452 ± 0.026	0.344 ± 0.069
0.50	99.50	0.341 ± 0.052	0.186 ± 0.042
0.10	99.90	0.300 ± 0.023	0.282 ± 0.059
0.05	99.95	0.255 ± 0.033	0.185 ± 0.053
0	100	0.113 ± 0.016	0.131 ± 0.014
BSA		0.045 ± 0.040	0.036 ± 0.024
milk protein		0.075 ± 0.032	0.078 ± 0.027

Sensitivity of MAb 5E12 for MBM Detection in Mixed Animal Feed Using the ELISA. The sensitivity of MAb 5E12 in detecting MBM was evaluated by testing 0−5% of MBM mixed in animal feed using an ELISA (**Table 1**). The reproducibility of the assay was confirmed by replicating three times ($p > 0.05$). The assay was performed in duplicate and replicated three times. The antibody diluted at 100- and 250-fold showed relatively strong affinity with MBM. Indeed, MAb 5E12 diluted to 100-fold was able to detect MBM down to 0.05% in animal feed. When the ELISA signal was plotted against the tested MBM content, a linear response was obtained from both the 100- and 250-fold-diluted 5E12 (**Figure 3**).

Effect of Heat Treatment on the Reactivity of Smooth Muscle with MAb 5E12. The effect of the heat treatment on the recognition of MBM by MAb 5E12 was evaluated using an ELISA by testing the antibody against bovine smooth muscle extracts autoclaved at 130 °C for 10, 30, 60, and 120 min (**Figure 4**). MAb 5E12 did not react with extract of non-heat-treated smooth muscle. However, its affinity toward smooth

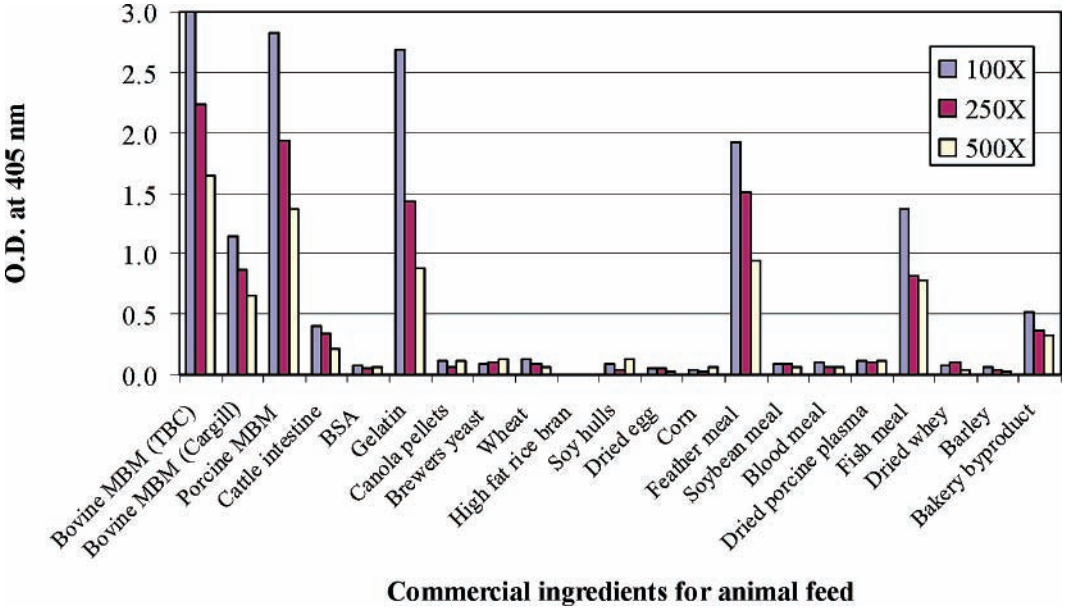

Figure 2. Cross-reactivity of 5E12 at 100-, 250-, and 500-fold dilutions against ingredients used for commercial animal feed as determined by the ELISA.

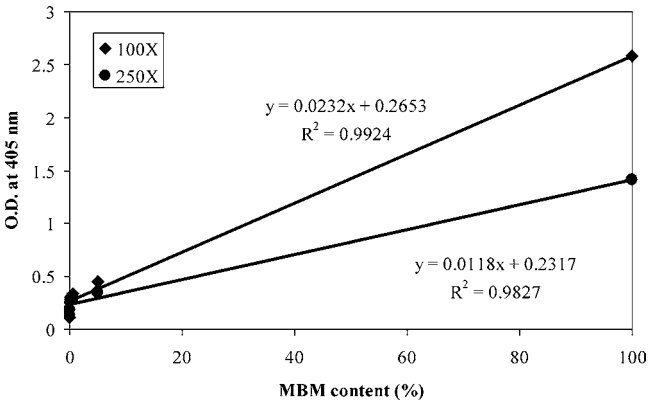

Figure 3. Calibration curves of MAb 5E12 at 100- and 250-fold dilutions in detecting MBM mixed in animal feed at various contents as determined by ELISA.

muscle was greatly enhanced when the sample was subject to heat treatment. Indeed, MAb 5E12 showed a slightly lower signal with the smooth muscle autoclaved at 130 °C for 30 min than with MBM. Its reactivity remained unchanged after the smooth muscle samples were autoclaved for up to 2 h. A similar trend was observed with the diluted MAb 5E12 at 100-, 250-, 500-, and 1000-fold dilutions.

Immunolabeling of Heat-Treated Bovine Intestine with 5E12. Because MAb 5E12 extraordinarily showed a strong affinity both with MBM and with autoclaved smooth muscles on ELISA, its binding to heat-treated smooth muscles was confirmed using an immunohistochemistry technique. Although none of the antibodies reacted with the freshly fixed bovine intestine tissues, MAb 5E12 gave very strong and specific labeling with the inner circular muscle layer of the tissue (**Figure 5A**,**B**). The labeling of 5E12 was seen to consist of a dense speckling in a sort of spiderweb network within the smooth muscle. There also appeared to have been some labeling of the muscle sheath of connective tissue around the muscle bundles (arrows in **Figure 5A**). The strong binding affinity of the antibody was clearly shown (**Figure 5B**). The controls run on the similar sections did not have this labeling with MAb 5E12. The positive MAb control, a mouse anti-acetylated tubulin antibody, labeled at the bases of the smooth muscle bundles.

DISCUSSION

Immunological methods are based on antibody recognition to specific antigens, and the approaches are generally rapid and easy to perform (*18*). It has been suggested that immunochemical assays can be considered as screening methods for detection of MBM in terms of practicability, if sensitivity and specificity requirements are fulfilled (*3*). The ELISA technique has been used for speciation of cooked and autoclaved meats by raising antibodies against heat-stable antigens; several commercial kits have been developed for the species identification of adulterated meats (*18*−*21*). These assay systems have been applied to evaluate the appropriate heat treatment of meat meal (*22*).

Limitations were observed in applying ELISA for the detection of MBM due to protein hydrolysis resulting from high temperature and pressure treatment (*23*). It was demonstrated that the detection rate of the assay system was greatly dependent on the temperature used for product treatment, even though the antibodies used for the tests were raised against heat-stable proteins (*22*). The sensitivity of the assays was significantly reduced when the test samples were heat treated at 133 °C. The assay system in detecting MBM was effective only when the processing conditions used for production did not meet the requirements of the European legislation (*24*). Therefore, there exists a need to develop antibodies that can detect MBM that had been subjected to the standard rendering conditions at 133 °C for 20 min and at the pressure of 300 kPa as regulated by the European Commission (*22*, *25*).

A heat-stable biomarker, *h*-caldesmon, was identified to produce antibodies for detection of MBM in our previous study (*26*). *h*-Caldesmon is a regulatory protein and is present in all types of smooth muscle but absent from cardiac and skeletal muscle (*27*). Its molecular weight was estimated on SDS-PAGE at 120000−150000. A peculiar property of the α-helical content in caldesmon is attributed to its heat stability (*28*). This enables the protein to refold in solution after several minutes of boiling. Therefore, this unique property is used extensively for the rapid purification of caldesmon (*29*, *30*). Clark et al. (*31*) reported that *h*-caldesmon is present in bovine smooth muscle at a relatively high concentration. We also confirmed the presence of *h*-caldesmon in MBM and its heat stability in autoclaved smooth muscles (*26*). In this study, the purified *h*-caldesmon

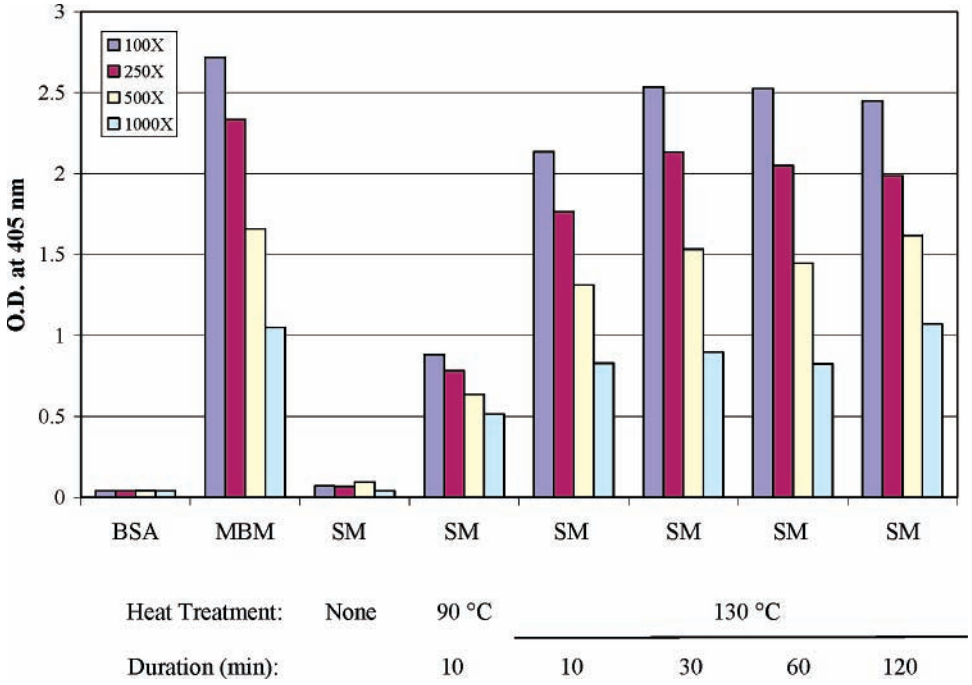

Figure 4. Affinity of MAb 5E12 at 1:100, 1:250, 1:500, and 1:1000 against the non-heat-treated and autoclaved bovine smooth muscles (SM) on ELISA. BSA and BMB were included as controls.

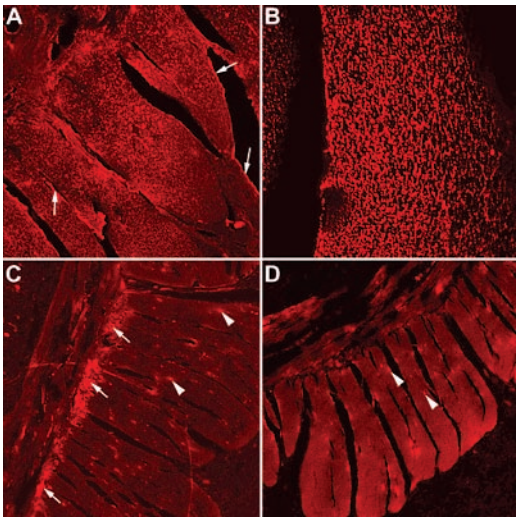

Figure 5. Labeling of MAb 5E12 on cattle intestine that had been autoclaved and then fixed with paraformaldehyde: (**A**) low magnification (20× objective); (**B**) high magnification (60× oil immersion objective); (**C**) positive control labeled with a mouse monoclonal anti-acetylated tubulin antibody; (**D**) negative control labeled with a mouse monoclonal anti-small cardioactive peptide. Fluorescent images were captured using a Bio-Rad MRC-1000 laser scanning confocal microscope equipped with a krypton–argon laser. (Figure is reproduced here at 75% of its original size.)

from bovine smooth muscle was identified as a better immunogen than MBM in producing antibodies for the development of immunoassay.

The produced MAb 5E12 showed a unique characteristic in detecting MBM and autoclaved smooth muscle. MAb 5E12 showed a stronger affinity against MBM than the heat-treated smooth muscle at 90 °C for 10 min. Subsequently, we found that heat treatment plays an important role in enhancing the affinity of the smooth muscle with the antibody. MAb 5E12 showed an almost similar reactivity with MBM and smooth muscles autoclaved at 135 °C, which is similar to the condition used for rendering MBM (**Figure 4**). This strong affinity of 5E12 was further confirmed by immunolabeling of the antibody onto autoclaved smooth muscle (**Figure 5**). Therefore, we postulated that the structural changes of proteins resulting from the rendering process might be associated with the exposure of epitope in the smooth muscle, thus promoting the binding of MAb 5E12 onto the rendered proteins.

The FDA's feed regulation listed MBM, meat meal, bone meal, blood meal, tankage, and offal as prohibited products (*7*). After the first outbreak of BSE in the United States in December 2003, the FDA also expanded the ban on cattle feed to include cow blood, chicken wastes, poultry litter, and plate waste (*32*). However, gelatin, milk products, and protein products from other species, such as porcine and equine, are exempt from this regulation. Although MAb 5E12 did not react with animal feed, other plant proteins, and milk products, it cross-reacted with gelatin and the related products. This antibody also reacted with both the bovine and porcine MBM. Our study also showed the typical limitation of immunoassay resulting from the lack of species-specificity of MAb (*11*, *33*, *34*). To improve the assay system, we are directing research toward the development of species-specific antibodies by using the species-specific domains of the biomarker as an immunogen.

Despite cross-reactivity, MAb 5E12 showed the potential possibility for use to screen the presence of MBM in animal feed and for development of a rapid and quantitative detection method for MBM in the field. The antibody was sensitive enough in detecting MBM contaminated in animal feed at $<0.05\%$ compared to other reported antibodies. This antibody also showed a linear response in detecting different amounts of MBM. Tested results were reproducible, which is critical in developing the assay system. In general, the sensitivity of the reported ELISA using polyclonal antibody was 1−5% of MBM in animal feed (*11*). The antibody raised by Chen et al. (*34*) against a heat-stable protein, troponin I, had detection limits of 0.3−2% of ruminant meat meal in feed samples and 0.5% of MBM in animal feed. Therefore, the sensitivity of our MAb

5E12 meets the requirement of 0.1% of MBM in animal feed suggested by the FDA (*11*).

In conclusion, *h*-caldesmon was suitable for MAb development for use as an analytical assay method for the detection of MBM in animal feed. Among the tested MAbs, 5E12 was identified as the best antibody. This antibody was able to differentiate MBM from most of the ingredients used for commercial animal feed production and strongly binds onto rendered bovine smooth muscles. This antibody also sensitively and quantitatively detects MBM mixed in animal feed at 0.05%.

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