Production and Characterization of a Monoclonal Anti-anti-idiotype Antibody against Fumonisin B_1

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A monoclonal anti-anti-idiotype antibody (mAb3) against fumonisin B_1 (FmB1) was produced from the hybridoma cell line 7C7F4, which was generated by the fusion of P3/NS-1/1-AG4-1 myeloma cells with spleen cells isolated from a Balb/c mouse that had been immunized with the Fab fragments of affinity-purified anti-idiotype antibodies. The mAb3 belongs to the immunoglobulin M, kappa light chain. A direct competitive enzyme-linked immunosorbent assay (dc-ELISA) and an indirect competitive ELISA (idc-ELISA) were established for antibody characterization and toxin analysis. In an idc-ELISA using FmB1—ovalbumin (OVA) as the coating antigen, the concentrations causing 50% inhibition of binding (IC_{50}) of mAb3 to the solid-phase FmB1—OVA by free FmB1, FmB2, and FmB3 were found to be 75, 95, and 450 ng/mL, respectively. In the dc-ELISA, the concentration causing IC_{50} of FmB1—horseradish peroxidase to the solid-phase mAb3 by free FmB1 was found to be 233 ng/mL. Analysis of 12 samples naturally contaminated with fumonisins with mAb3-based idc-ELISA and polyclonal-based dc-ELISA showed a good correlation between these two methods with a correlation coefficient of 0.76 at $p < 0.02$. The linear regression slope was found to be y[polyclonal ELISA] = 0.87x[mAb3 ELISA] − 52 ppb.

Keywords: *Mycotoxin; fumonisin; ELISA; anti-idiotype; antibodies*

INTRODUCTION

Fumonisins are a group of toxic metabolites produced primarily by *Fusarium moniliforme*, one of the most common fungi colonizing corn throughout the world. Fumonisin B_1 (FmB1, diester of propane-1,2,3-tricarboxylic acid of 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane), the most common naturally occurring fumonisin in this group of mycotoxins, has been found to be a potent cancer promoter as well as an etiological toxic agent responsible for the equine leukoencephalomalacia (ELEM) disease (Norred, 1993; Riley and Richard, 1992; Thiel et al., 1992) and pulmonary edema in swine (Colvin and Harrison, 1992; Osweiler et al., 1992). Preliminary reports on the carcinogenicity of FmB1 in rats (Gelderblom et al., 1991; Thiel et al., 1992) and recent reports on the worldwide occurrence of this group of mycotoxins in foods and feeds have prompted an intensive study on this group of mycotoxins (Dutton, 1996; Jackson et al., 1996).

In view of their potential hazard to human and animal health, extensive research has been conducted to develop more efficient methods for fumonisin determination. Several high-performance liquid chromatography (HPLC) (Bennet and Richard, 1994; Holcomb et al., 1993; Murphy et al., 1993; Shephard et al., 1990; Thakur and Smith, 1996; Thiel et al., 1992; Ware et al., 1993; Wilson et al., 1998) methods with a high degree of sensitivity and accuracy have been developed. However, these methods generally require extensive sample cleanup and precolumn derivatization. Immunochemical methods have gained wide application for mycotoxin analysis during the past few years (Chu, 1996a,b; Pestka, 1988; Trucksess, 1998; Scott and Trucksess, 1997). With the availability of antibodies against FmB1 and an increase in the use of immunochemical methods for detecting fumonisins (Azcona-Olivera et al., 1992a,b; Fukuda et al., 1994; Usleber et al., 1994; Yeung et al., 1996; Yu and Chu, 1996), there is a great demand for specific antibodies and related immunochemical reagents for the assay.

An alternative approach for preparing immunochemical reagents is through the generation of anti-idiotype antibodies (Kennedy et al., 1987; Nisonoff, 1991). Some anti-idiotype antibodies have been shown to mimic the biological functions of the hapten effectively (Chanh et al., 1992; Nisonoff, 1991). They can also be used as antigen surrogates in the immunoassays as well as to generate anti-anti-idiotype antibodies which have specificity similar to that of the original antibody (Chu et al., 1995; Hsu and Chu, 1994; Liu et al., 1996; Shestowsky et al., 1993). With the availability of anti-idiotype and anti-anti-idiotype antibodies as the immunoassay reagents, it is possible to avoid the use of potentially toxic markers in the assay system and replace the toxin conjugates with an immunogen for antibody production.

Polyclonal anti-idiotype antibodies for FmB1 (pAb2) were generated in rabbits after immunizatiomn with affinity-purified monoclonal antibodies (mAb). Murine polyclonal anti-anti-idiotype antibodies against fumonisin B1 (pAb3) were also produced in the ascites of Balb/c mice immunized with affinity-purified pAb2 (Chu

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et al., 1995). However, wide application of pAb3-based ELISA for FmB1 is limited because of the limited amount of pAb3 produced in the ascites. Thus, an attempt was made to generate a monoclonal anti-anti-idiotype antibody for fumonisins (mAb3) using the Fab fragment of affinity-purified pAb2 as an immunogen. Details for the production and characterization of the mAb3 and its use in immunoassays of FmB1 are reported herein.

MATERIALS AND METHODS

Materials. Fumonisins B_1, B_2, and B_3, corn samples, and monoclonal antibodies P2A5-3-F3 (mAb1) originally generated from a mouse immunized with FmB1−cholera toxin conjugate (FmB1−CT) were kindly supplied by Dr. Chris M. Maragos of the National Center for Agricultural Utilization Research (NCAUR) of the U.S. Department of Agriculture (USDA) (Peoria, IL). FmB1 was also kindly supplied by Dr. Robert M. Eppley and Dr. M. Trucksess of the Food and Drug Administration (Washington, DC). Polyconal antibodies against FmB1 (pAb1) were produced by immunizing rabbits with FmB1−KLH (Yu and Chu, 1996). FmB1−OVA was prepared by using glutaraldehyde in the cross-linking method as described by Yu and Chu (1996). FmB1−HRP was prepared according to the periodate method (Nakane and Kawaoi, 1974). Ovalbumin, Tween 20, normal mouse IgG, and pristane (2,6,10,14-tetramethylpentadecane, T-7640) were obtained from Sigma Chemical Co. (St. Louis, MO). Dithiothreitol (DTT) was obtained from United States Biochemicals (USB, Cleveland, OH). Immobilized papain gel (no. 20341), immobilized protein A (no. 20333, immunopure), AminoLink gel, and goat anti-mouse IgG plus IgM−peroxidase conjugate (ELISA grade) were obtained from Pierce Chemical Co. (Rockford, IL). Goat anti-rabbit IgG−HRP conjugate, horseradish peroxidase (HRP, ELISA grade), polyethylene glycol (PEG 1500), hypoxanthine (H), thymidine (T), and aminopterin (A) were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Activated CH-Sepharose 4B was obtained from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ). 3,3′,5,5′-Tetramethylbenzidine (K-Blue) was purchased from ELISA Technologies (Lexington, KY). Virus-free, 9−10-week-old, female Balb/c mice were obtained from Harlan Sprague Dawley (Madison, WI). The murine myeloma cell line P3/NS-1/1-AG4-1 (NS-1) was obtained from American Type Culture Collection (Rockville, MD). All other chemicals and organic solvents used were of reagent grade or better.

Preparation of the Fab Fragment of pAb2 as Immunogen. pAb2 were obtained by immunizing rabbits with the mAb1 against FmB1−CT conjugate (Chu et al., 1995). The pAb2 were purified by ammonium sulfate precipitation and affinity chromatography on a column conjugated with mAb1 as described by Chu et al. (1995). For generating mAb3 specific to fumonisins, the Fab fragment of the purified pAb2 (2.9 mg) was prepared by digesting the antibody with an immobilized papain gel according to the supplier's instructions (Pierce, IL). In general, 0.5 mL of papain gel was equilibrated and washed twice with 4 mL of digestion buffer [(DB), 20 mM NaH_2PO_4 plus 20 mM dithiothreitol (instead of 20 mM cysteine hydrocholide) plus 10 mM EDTA-Na_4, pH 7.0]. After centrifugation at 2000 rpm (1600*g*) for 2 min, the gel was suspended in 0.5 mL of DB. The affinity-purified pAb2 (2.9 mg) obtained above, in 0.5 mL of DB, were incubated with 0.5 mL of papain gel at 37 °C on a shaker for 5 h. The reaction was terminated by the addition of 1.5 mL of 0.01 M Tris-HCl (pH 7.5) and centrifuged to remove the papain gel. The supernatant solution was dialyzed against 2 L of 0.01 M phosphate buffer (pH 7.5) and stored at −20 °C. Further purification of the Fab fragment of pAb2 was achieved by passing the digested solution through a protein A column (2 mL gel size) according to the manufacturer's instructions (Pierce, IL). Typically, the dialyzed and lyophilized preparation (2.5 mg) from papain digestion was redissolved in 1 mL of 0.01 M phosphate buffer (pH 7.5) containing 0.15 M NaCl (PBS), applied to the prepacked column, and incubated at room temperature for 1 h. The unbound Fab fragment (∼2.0 mg) was obtained by washing the column with 15 mL of PBS after the incubation.

Production of a Monoclonal Anti-anti-idiotype Antibody (mAb3) Specific to FmB1. Protocols for the production of mAb were similar to those described by Fan et al. (1988).

Immunization. For generating mAb3 antibodies, four female Balb/c mice (9−10 weeks of age) were each immunized with 40 μg of the Fab fragments of pAb2 in 0.2 mL of 0.01 M PBS that has been emulsified with an equal volume of Freund's complete adjuvant. Booster injections, at 40 μg of Fab fragments in 0.3 mL of PBS containing no adjuvant for each mouse, were made every week beginning 4 weeks after the initial immunization. Blood samples were removed from tails at weekly intervals after each booster injection. An indirect ELISA as described below was used to determine the antibody titer in the serum.

Fusion and Cloning. The mouse (no. 1) with the highest antibody titer (8 weeks after the initial immunization and three booster injections) was selected for fusion. The antiserum titer was defined as the reciprocal of the antiserum dilution that gives an absorbance of 1.0 in the indirect ELISA. Four days before fusion, the mouse was primed with 50 μg of immunogen (twice with 20 μg via ip injection and once with 10 μg via iv injection). The mouse was sacrificed by cervical dislocation. The whole spleen was aseptically removed and mashed with a glass pestle through a cell dissociation sieve to produce a single-cell suspension, which was then combined with 10^7 myeloma cells. The cells were centrifuged (1000*g*), suspended in 0.2 mL of HT medium, and then fused by the addition of 1 mL of PEG 1500 in 1 min. After fusion, the cells were pelleted, suspended in HAT medium plus normal mouse erythrocytes to a final concentration of 0.5%, and plated into 96-well tissue culture plates (Corning plate 25860; Corning, NY). The colonies were fed every fifth day with freshly prepared HAT medium. When the colonies reached at least half-confluency in the well, hybridomas were screened for specific antibodies against FmB1 using an indirect ELISA described below. Wells containing positive cells were cloned by the limiting dilution method into 96-well tissue culture plates (Oi and Herzenberg, 1980).

Production of Ascites Fluid. Female Balb/c mice, 10 weeks old, were injected (ip) with 0.5 mL of 2,6,10,14-tetramethylpentadecane (pristane) 7 days before receiving an ip injection of 2×10^6 cells of hybridoma cell line suspended in DMEM. Ascites fluid developed 2−3 weeks after the injection of the cells and was collected every other day for ∼8 days. The ascites fluid was centrifuged at 7000 rpm (5900*g*) for 5 min to remove cell debris and then frozen at −20 °C. Purification of mAb3 was achieved by precipitation of the supernatant solution with saturated $(NH_4)_2SO_4$ twice and passing through an affinity column armed with an FmB1-gel, similar to the purification procedure for mAb1 (Chu et al., 1995).

Characterization of mAb3 Antibodies. *Determination of Isotype.* A commercially available mouse immunoglobulin isotype identification kit (Boehringer Mannheim, Indianapolis, IN) was used to determine the isotype of mAb3 according to the manufacturer's protocol.

mAb3-Based Indirect Competitive ELISA (idc-ELISA) for Characterization of Antibody. An idc-ELISA was used to characterize mAb3. Briefly, each well of a microtiter plate (plate 2-69620; Nunc, Roskilde, Denmark) was coated with 100 μL of FmB1−OVA (1 μg/mL in PBS, pH 7.5). After overnight incubation at 4 °C, the wells were washed four times with PBS−Tween buffer (0.35 mL per well; 0.05% Tween 20 in 0.01 M PBS). This was followed by incubation with 0.17 mL of 0.1% gelatin in 0.01 M PBS (blocking buffer) at 37 °C for 30 min. The plate was washed again as described above, and then mouse antiserum (50 μL/well, different dilutions in PBS), culture supernatant (50 μL/well) or purified mAb3 (50 μL/well, 10 μg/mL in PBS, 0.01 M, PH 7.5) together with 50 μL of FmB1, FmB2, and FmB3 at different concentrations (1−1000 ng/mL) was added. After incubation at 37 °C for 1 h, the plate was washed, and 0.1 mL of goat anti-mouse IgG plus IgM−peroxidase conjugate (1:10000 dilution in PBS) was added. The plate was incubated at 37 °C for 45 min, washed four times

with PBS−Tween buffer, and followed by the addition of 0.1 mL of K-Blue substrate solution (1 mM 3,3′,5,5′-tetramethylbenzidine and 3 mM H_2O_2 per liter of potassium citrate buffer, pH 3.9; a premixed solution supplied by ELISA Technologies). Ten minutes after incubation at room temperature in the dark, the reaction was terminated by adding 0.1 mL of 1 N HCl to each well. Finally, the plate was analyzed at dual endpoint absorbances of 450−650 nm using an automatic ELISA reader (THERMOmax microplate reader, Molecular Devices Co., Menlo Park, CA).

mAb3-Based dc-ELISA. The protocol for dc-ELISA was essentially the same as that we have described previously for aflatoxin B_1 (Fan and Chu, 1984), except that the purified mAb3 diluted in 0.01 M PBS was used (25 μg/mL with 0.1 mL/well). After incubating at 4 °C overnight, the plate was washed again with PBS−Tween (0.35 mL/well; 0.05% Tween 20 in 0.01 M PBS) followed by blocking with BSA−PBS (0.17 mL/well; 0.1% BSA in 0.01 M PBS) at 37 °C for 30 min. The plate was washed again with PBS−Tween four times, followed by incubation of FmB1 (0.05 mL/well) at various dilutions or blank buffer together with the FmB1−HRP conjugate (1:200 dilution, 10 μg/mL in 0.01 M PBS, 0.05 mL/well) at 37 °C for 50 min. The plate was washed four times with PBS−Tween buffer, and color development was conducted as described above.

Analysis of Fumonisins in Naturally Contaminated Samples with mAb3-Based and pAb1-Based ELISA. Twelve naturally contaminated samples (2 starch and 10 corn-based foods) that had been previously analyzed for FmB1 with HPLC by scientists of the NCAUR of the USDA (Peoria, IL) (Bennett and Richard, 1994) and for fumonisins with ELISA (Yu and Chu, 1996) were used to test the efficiency of mAb3-based idc-ELISA for fumonisins. Briefly, 10 g of ground samples supplied by Dr. Chris M. Maragos was shaken with 100 mL of CH_3CN/H_2O (1:1, v/v) at 4 °C overnight. After the suspension was allowed to settle, the sample extracts were subjected to a C_{18} reverse-phase cartridge cleanup. Typically, 3 mL of the extracts together with 9 mL of acidified distilled water (pH 4.0) was added to a C_{18} reversed-phase Sep-Pak cartridge that had been washed with 2 mL of CH_3CN and 4 mL of distilled water in sequence. The cartridge was then washed with 4 mL of acidified distilled water (pH 4.0) followed by 4 mL of CH_3CN/H_2O (15:85, v/v). Fumonisins were eluted from the column with 4 mL of CH_3CN/H_2O (7:3, v/v), diluted with PBS, and then subjected to ELISA analysis.

RESULTS

Characterization of Antiserum from Mice Immunized with pAb2. For the generation of mAb3 for FmB1, four Balb/c mice were each immunized with purified Fab fragments of pAb2. After three booster injections, the antibody titers peaked at the eighth week after the initial immunization. Results of typical ELISA titration curves of the eighth week antiserum are shown in the inset of Figure 1. The specific binding of antiserum against FmB1 was determined using idc-ELISA in which free FmB1 was added to the wells of the ELISA plate as the competitor. Results indicated that the antiserum from mice not only bound to the FmB1−OVA coated on the wells but also could be displaced by various concentrations of free FmB1. Among four mice immunized with the Fab fragment, serum obtained from mouse 1 had the highest titer, and the binding of the antiserum to the solid-phase FmB1−OVA was also capable of being displaced by free FmB1. For example, when the antiserum of mouse 1 was used in the idc-ELISA, the concentration causing 50% inhibition (IC_{50}) of binding of the antiserum to the solid-phase FmB1−OVA by FmB1 was found to be 472 ng/mL (Figure 1). Thus, spleen cells obtained from mouse 1 were used for fusion in generating mAb3.

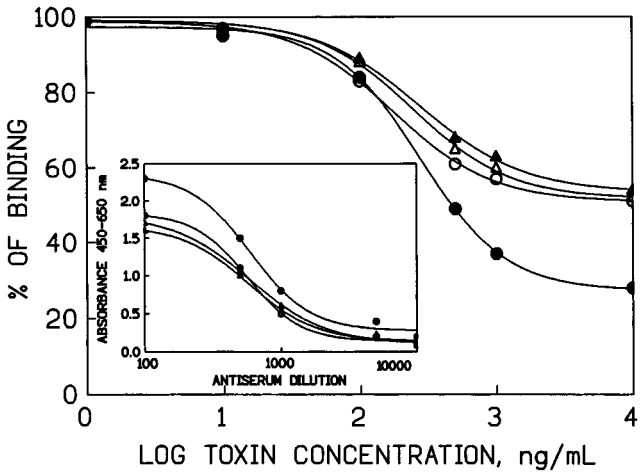

Figure 1. Determination of serum titers of mice 1 (●), 2 (○), 3 (▲), and 4 (△) in an idc-ELISA. The microtiter plate wells were each coated with 0.1 mL of FmB1−OVA (1 μg/mL). The binding of 0.05 mL of antiserum (1:200 dilution in PBS) to the solid-phase FmB1−OVA in the presence of FmB1 (0.05 mL/well) standard toxin was determined by goat anti-mouse IgG−IgM HRP conjugate (1:10000 dilution, 0.1 mL/well). The antibody titers for each mouse are given in the inset. Each data point represents the mean of triplicate determinations.

Production of a Monoclonal Anti-anti-idiotype Antibody (mAb3) Specific to FmB1. Fourteen days after fusion, >630 wells of 10 tissue culture plates (total of 960 wells) had cell colony growth. Initial screening of the hybridoma culture supernatants showed 15 wells containing stable master cell lines producing antibodies that were reactive with FmB1−OVA coated on the ELISA plate. Subsequent screening of these clones with idc-ELISA showed that only 2 wells of cells produced antibodies capable of binding with solid-phase FmB1−OVA, and such binding could also be displaced by free FmB1. The hybridomas in the well, 7C7, producing antibodies with the highest binding with FmB1 were selected and then cloned by the limiting dilution method. Finally, cell line 7C7F4 showing stable growth and steady antibody production was used for the production of antibody in mouse ascites fluid. The ascites fluids generated from 7C7F4 clone were purified through ammonium sulfate precipitation, subjected to an affinity column armed with FmB1, and then used for further characterization.

Characterization of the mAb3 Antibodies. The isotype of mAb3 generated by 7C7F4 was found to be immunoglobulin M (IgM), kappa-light chain. The specificity of mAb3 was determined with both the idc- and dc-ELISAs. In the idc-ELISA, FmB1−OVA was coated to the wells of the ELISA plate to serve as solid-phase antigen; results for the inhibition of binding of mAb3 to FmB1−OVA by various concentrations of fumonisins are shown in Figure 2. The IC_{50} valuesof binding of mAb3 to the solid-phase FmB1−OVA by free FmB1, FmB2, and FmB3 were found to be 75, 95, and 450 ng/mL, respectively. The relative cross-reactivities of mAb3 to FmB1 (=100), FmB2, and FmB3 were calculated to be 100, 79, and 17, respectively. In the mAb3-based dc-ELISA, the concentration causing 50% inhibition of binding of mAb3 to FmB1−HRP by FmB1 was found to be 233 ng/mL (Figure 3).

Comparison of Various Antibodies Specific to FmB1 in an idc-ELISA. The efficacies of mAb3 generated in the present study and other anti-FmB1 antibodies generated previously in our laboratory (Chu et al.,

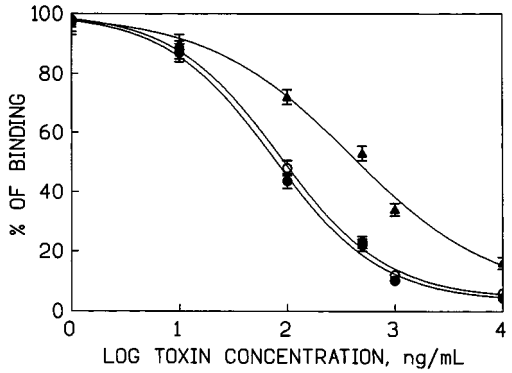

Figure 2. Cross-reactivity of mAb3 with FmB1 (●), FmB2 (○), and FmB3 (▲) in an idc-ELISA. The microtiter plate wells were each coated with 0.1 mL of FmB1−OVA (1 μg/mL). The binding of 0.05 mL of mAb3 (10 μg/mL) to the solid-phase FmB1−OVA in the presence of FmB1, FmB2, and FmB3 (0.05 mL/well) standard toxin was determined by goat anti-mouse−HRP conjugate (1:10000, 0.1 mL/well). Each data point represents the mean of triplicate determinations and standard deviations. The absorbance of the control, A_0, with no toxin present, was 1.2.

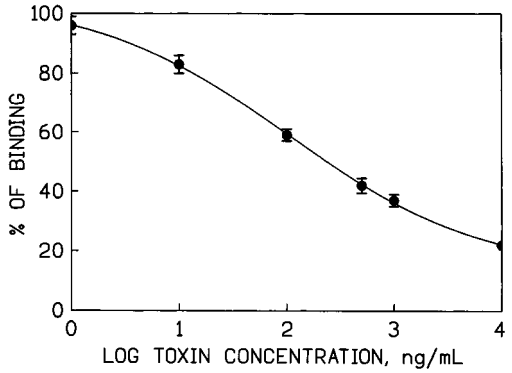

Figure 3. Standard curve of FmB1 (●) in an mAb3-based dc-ELISA. Microtiter plate wells were each coated with 0.1 mL of mAb3 (25 μg/mL). Fifty microliters of FmB1−HRP (1:200, 10 μg/mL) plus 0.05 mL of standard toxin was used in each assay. The concentration causing IC_{50} of FmB1−HRP to the antibodies by FmB1 in the ELISA was 233 ng/mL. Each data point represents the mean of triplicate determinations and standard deviations. The absorbance of the control, A_0, with no toxin present, was 0.8.

Table 1. Comparison of the Concentration of Free Fumonisins Causing IC_{50} of Anti-FumB_1 (pAb1, mAb1) and Anti-anti-idiotype (pAb3, mAb3) Antibodies in the idc-ELISA[a]

	IC_{50} concn (ng/mL)		
antibody type	FmB1	FmB2	FmB3
FmB1-pAb1[b]	0.45	0.62	25
FmB1-mAb1[c]	140	150	nd[f]
FmB1-pAb3[d]	190	260	nd
FmB1-mAb3[e]	75	95	450

[a] FmB1−OVA was used as the coating antigen in of all the tests. [b] FmB1-pAb1: pAb1 against FmB1−KLH conjugate (Yu and Chu, 1996). [c] FmB1-mAb1: mAb1 against FmB1−CT conjugate (Chu et al., 1995), which is the antibody in generating pAb2 used in the present work. [d] FmB1-pAb3: polyclonal antibodies generated in the ascites fluid of Balb/c mice after immunization with affinity-purified pAb2 as in footnote *c*. [e] FmB1-mAb3: monoclonal antibody generated in the present study. [f] nd, not detectable.

1995; Yu and Chu, 1996) for fumonisin analyses in the idc-ELISA are compared in Table 1. In the mAb1-based idc-ELISA in which FmB1−OVA served as the solid-phase antigen, the IC_{50} values of binding of mAb1 to FmB1−OVA by FmB1 and FmB2 were found to be 140 and 150 ng/mL, respectively; FmB3 was not detectable in this system. In the pAb3-based idc-ELISA, the IC_{50} values of binding of pAb3 to the solid-phase FmB1−OVA by free FmB1 and FmB2 were found to be 190 and 260 ng/mL, respectively. Thus, all of the antibodies, that is, mAb1, pAb3, and mAb3, showed the highest specificity for FmB1, less for FmB2, and least for FmB3.

Table 2. Comparison of the FmB1 in Naturally Contaminated Corn Samples Detected by mAb3-Based and pAb-Based ELISA and HPLC

		ELISA[a] (ng/g)		HPLC[b] (ng/g)
	sample tested	mAb3	pAb	FmB1
1	White Wings tortilla mix	609	716	584
2	NC yellow corn	889	558	326
3	white corn crete mills	433	382	274
4	Garver bag corn	361	538	358
5	Maseca masa	494	288	110
6	Quaker masa	228	40	<10
7	AZ corn	108000	63527	49800
8	corn	53275	22461	9800
9	grits yellow no. 5	298	108	84
10	grits yellow no. 6	18946	10713	9300
11	starch no. 1	205	18	<10
12	starch no. 2	469	372	312

[a] ELISA based on FmB1 standard. [b] HPLC was conducted by scientists of the NCAUR of the USDA using the method of Bennent and Richard (1994); all of the samples were subjected to a cleanup before analysis.

Analysis of Fumonisins in Samples with mAb3-Based and pAb1-Based ELISA. To test the effectiveness of mAb3-based idc-ELISA, 12 naturally contaminated samples, including corn and starch, that had been previousely analyzed with HPLC by scientists at the NCAUR of the USDA (Peoria, IL) were analyzed with both the mAb3-based idc-ELISA and the pAb1-based dc-ELISA. Results of these analyses are presented in Table 2. A wide range of levels of fumonisins was found in these samples by the mAb3-based ELISA. As low as 205 ppb of FmB1 was found in one of the starch samples. On the other hand, two samples contained fumonisins at levels >50 ppm. Although a positive correlation between the data obtained from the mAb3-based and pAb1-based dc-ELISAs was obtained, data from the mAb3-based ELISA were higher. The correlation coefficient of a linear regression between these two ELISAs was found to be 0.76 at $p < 0.05$ with a regression slope of y[pAb ELISA] = 0.87x[mAb3 ELISA] − 52 ppb.

DISCUSSION

Recent development for the wide application of immunoassay for mycotoxins, including FmB1, has led to increased demand for immunochemical reagents. Anti-idiotype antibodies and anti-anti idiotype antibodies provide an alternative approach for obtaining these reagents. In the present study, we have demonstrated that the purified Fab fragments of pAb2 specific to anti-FmB1-mAb1, a monoclonal antibody generated using FmB1−CT as immunogen, were an effective immunogen in generating mAb3. The mAb3 not only mimics the specificity and the binding characteristics of mAb1 but also shows the potential to be used for analytical purposes. These data suggest that some populations of the pAb2, namely Ab2β, bear an internal image of the FmB1 for producing Ab3. However, Ab2β is known to represent only a very small fraction of the total heterogeneous Ab2 population, which is composed of Ab2α,

Ab2β, and Ab2γ (Jern et al., 1974; Chen et al., 1991). Therefore, after the first attempts to select any positive cell line out of ~400 wells of hybridomas from a previous fusion (data not shown) failed, only 2 of 600 wells of hybridomas showing the ability to produce stable antibodies with high affinity to FmB1 were selected after fusion with the spleenocytes from one of the four mice immunized with the Fab fragment of pAb2.

From the IC_{50} values of various antibodies in the idc-ELISA, it is apparent that mAb3 have characteristics similar to those of the original mAb1. The sensitivity of mAb3-based idc-ELISA for FmB1 and FmB2 (IC_{50} values of 75 and 95 ng/mL, respectively) developed in the present study is comparable or slightly better than that of the original mAb1-based ELISA (140 and 150 ng/mL for FmB1 and FmB2, respectively). Both mAb1 and mAb3 showed similar specificities toward FmB1 and FmB2 but less specificity toward FmB3 in idc-ELISAs. However, it is necessary to purify mAb3 through an FmB1 affinity column armed with toxin FmB1 to achieve its maximal specificity and sensitivity.

We have previously demonstrated that purified Fab fragments of pAb2 elicited by anti-FmB1−CT mAb1 were effective immunogens for generating polyclonal anti-anti-idiotype antibodies (pAb3) in mice ascites fluids (Chu et al., 1995). The IC_{50} values of mAb3 for FmB1 and FmB2 obtained in the present study are lower than those of pAb3 (190 and 260 ng/mL for FmB1 and FmB2, respectively) in an idc-ELISA, suggesting mAb3 are more sensitive than pAb3 in fumonisin analyses. Because of the unlimited supply of mAb3, it is possible to use mAb3 and Ab2 as alternative immunochemical reagents to avoid potentially toxic toxin−enzyme conjugates in the fumonisin assay system and to replace toxin conjugates as immunogens for antibody production.

Although we found a good correlation between the data obtained from the mAb3-based idcELISA and those from the pAb1-based dc-ELISAs for the analysis of naturally contaminated samples (correlation coefficient of 0.76, $p < 0.05$), data obtained from mAb3-based ELISA were generally higher than those measured by the pAb1-based ELISA and HPLC, especially in the heavily contaminated samples, that is, samples 7, 8, and 10. Nevertheless, these data show an improvement over other mAb-based studies, in which the ELISA data sometimes were 2−3-fold higher than those obtained from HPLC (Murphy et al., 1993; Pestka et al., 1994; Usleber et al., 1994). Because the HPLC method determined only the FmB1 concentrations in samples, the high cross-reactivity of mAb3 with FmB1 analogues, such as FmB2, in the idc-ELISA may partially contribute to the higher analytical results in mAb3-based ELISA than in HPLC. Linear regression analysis between mAb3-based ELISA and HPLC showed that the correlation coefficient was 0.64 (y[HPLC] = 0.57x[mAb3 ELISA] − 25 ppb) with $p < 0.1$. The weaker correlation between the mAb3-based ELISA and HPLC data than that between pAb-based ELISA and HPLC may be also caused by a higher background absorbance in the mAb3-based ELISA and a lower affinity of mAb3 to fumonisins than that of pAb1. From a comparison of the mAb-based ELISA with the pAb-based ELISA for fumonisins, the pAb-based ELISA appears to be more sensitive and has fewer matrix inteference problems than the monoclonal antibody-based ELISA. Unless a high-affinity mAb for fumonisin is obtained, the matrix interference problem cannot be overlooked in such assays.

The present study has demonstrated that monoclonal anti-anti-idiotype antibody could be generated and the mAb3 could be used for diagnostic and possible therapeutic purposes. In addition to practical applications, an understanding of the structure of mAb3 could have some academic interest as well. The nucleotide and deduced amino acid sequences of the variable regions of the heavy (V_H) and light (V_L) chains for the anti-FmB1−KLH mAb1 have been determined in our laboratory (Yu, 1997). The variable region of a single-chain (scFv) DNA fragment of anti-FmB1−CT mAb1 that had been cloned into an M13 phagemid vector has also been expressed in *Escherichia coli* by Zhou et al. (1996). It would be interesting to know the sequences of the V_H and V_L regions of mAb3 of the present study so that the configurations of motifs for the binding of FmB1 with both antibodies could be compared. Such knowledge would be very useful for future cloning work to design high-binding antibodies against fumonisins.

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LITERATURE CITED

Azcona-Olivera, J. I.; Abouzid, M. M.; Plattner, R. D.; Norred, W. P.; Pestka, J. J. Generation of antibodies reactive fumonisins B1, B2, and B3 by using cholera toxin as the carrier-adjuvant. *Appl. Environ. Microbiol*. **1992a**, *58*, 169−173.

Azcona-Olivera, J. I.; Abouzid, M. M.; Plattner, R. D.; Pestka, J. J. Production of monoclonal antibodies to the mycotoxins fumonisin B1, B2, B3. *J. Agric. Food Chem*. **1992b**, *40*, 531−534.

Bennett, G. A.; Richard, J. L. Liquid chromatographic method of analysis for the naphthalene dicarboxyaldehyde derivative of fumonisins. *J. AOAC Int*. **1994**, *77*, 501−506.

Chanh, T. C.; Kennedy, R. C.; Hewetson, J. F. Anti-idiotype vaccines in toxicology. *Int. J. Clin. Lab. Res*. **1992**, *144*, 4721−4728.

Chen, Z.; Yang, N.; Kageshita, T.; Ferrone, S. Human high-molecular-weight melanoma-associated antigen mimicry by mouse anti-idiotypic monoclonal antibody TK7−371. *Cancer Res*. **1991**, *51*, 4790−4797.

Chu, F. S. Recent studies on immunoassays for mycotoxins. In *Immunoassays for Residue Analysis: Food Safety*; Beier, R. C., Stanker, L. H., Eds.; ACS Symposium Series 621; American Chemical Society: Washington, DC, 1996a; pp 294−313.

Chu, F. S. Immunochemical methods for fumonisins. In *Fumonisins in Food*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Publishing: New York, 1996b; pp 123−134.

Chu, F. S.; Huang, X.; Maragos, C. M. Production and characterization of anti-idiotype and anti-anti-idiotype antibodies against fumonisin B1. *J. Agric. Food Chem*. **1995**, *43*, 261−267.

Colvin, B. M.; Harrison, L. R. Fumonisins induced pulmonary edema and hydrothorax in swine. *Mycopathologia* **1992**, *117*, 79−82.

Dutton, M. F. Fumonisins, mycotoxins of increasing importance: their nature and their effects. *Pharmacol. Ther*. **1996**, *70*, 137−161.

Fan, T. S. L.; Chu, F. S. Indirect enzyme linked immunosorbent assay for detection of aflatoxin B1 in corn and peanut butter. *J. Food Prot.* **1984**, *47*, 263−266.

Fan, T. S. L.; Schubring, S. L.; Wei, R. D.; Chu, F. S. Production and characterization of a monoclonal antibody cross-reactive with most group A trichothecenes. *Appl. Environ. Microbiol.* **1988**, *54*, 2959−2963.

Fukuda, S.; Nagahara, A.; Kikuchi, M.; Kumagai, S. Preparation and characterization of antifumonisin monoclonal antibodies. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 765−767.

Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B1, in rat. *Carcinogenesis* **1991**, *12*, 1247−1251.

Holcomb, M.; Thompson, H. C.; Hankins, L. J. Analysis of fumonisin B1 in rodent feed by gradient elution HPLC using precolumn derivatization with FMOC and fluorescence detection. *J. Agric. Food Chem.* **1993**, *41*, 764−767.

Hsu, K. H.; Chu, F. S. Production and characterization of anti-idiotype and anti-anti-idiotype antibodies from a monoclonal antibody against aflatoxin. *J. Agric. Food Chem.* **1994**, *42*, 2353−2359.

Jackson, L., DeVries, J. W., Bullerman L. B., Eds. *Fumonisins in Food*; Plenum Publishing: New York, 1996; p 395.

Jerne, N. K. Towards a network theory of the immune system. *Ann. Immunol.* **1974**, *125*, 373−389.

Kennedy, R. C.; Zhou, E. M.; Lanford, R. E.; Chanh, T. C.; Bona, C. A. Possible role of anti-idiotype antibodies in the induction of tumor immunity. *J. Clin. Invest.* **1987**, *80*, 1217−1224.

Liu, B. H.; Yu, F. Y.; Chu, F. S. Anti-idiotype and anti-anti-idiotype antibodies generated from polyclonal antibodies against Microcystin-LR. *J. Agric. Food Chem.* **1996**, *44*, 4037−4042.

Murphy, P. A.; Rice, L. G.; Ross, P. F. Fumonisins-B1, B2 and B3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. *J. Agric. Food Chem.* **1993**, *41*, 263−266.

Nakane, P. K.; Kawaoi, A. Peroxidase-labeled antibody: a new method of conjugation. *J. Histochem. Cytochem.* **1974**, *22*, 1084−1091.

Nisonoff, A. Idiotypes: Concepts and applications. *J. Immunol.* **1991**, *147*, 2429−2438.

Norred, W. P. Fumonisins-mycotoxin-produced by *Fusarium moniliforme. J. Toxicol. Environ. Health* **1993**, *38*, 309−328.

Oi, V. T.; Herzenberg, L. A. Immunoglobulin-production hybrid cell lines. In *Selected Methods in Cellular Immunology*; Mishell, B. B., Shiigi, S. M., Eds.; Freeman: San Francisco, CA, 1980; pp 351−371.

Osweiler, G. D.; Ross, P. F.; Wilson, T. M.; Nelson, P. E.; Witte, S. T.; Carson, T. L.; Rice, L. G.; Nelson, H. A. Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. *J. Vet. Diagn. Invest.* **1992**, *4*, 53−59.

Pestka, J. J. Enhanced surveillance of foodborne mycotoxin by immunochemical assay. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 1075−1081.

Pestka, J. J.; Azcona-Olivera, J. I.; Platter, R. D.; Minervini, F.; Doko, B.; Visconti, A. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS, and HPLC. *J. Food Prot.* **1994**, *57*, 167−172.

Riley, R. T., Richard, J. L., Eds. Fumonisins: A current perspective and view to the future. *Mycopathologia* **1992**, *117*, 1−124.

Scott, P. M.; Trucksess, M. W. Application of immunoaffinity columns to mycotoxin analysis [review]. *J. AOAC Int.* **1997**, *80*, 941−949.

Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B1 and B2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077−2087.

Shestowsky, W. S.; Quilliam, M. A.; Sikorska, H. M. An idiotype-anti-idiotype competitive immunoassay for quantitation of okadaic acid. *Toxicon* **1992**, *30*, 1441−1448.

Spinks, C. A.; Wang, B.; Mills, E. N. C.; Morgan, M. R. A. Production and characterization of monoclonal anti-idiotype antibody mimics for the pyrethroid insecticides and herbicide paraquat. *Food Agric. Immunol.* **1993**, *5*, 13−25.

Thakur, R. A.; Smith, J. S. Determination of fumonisin B1 and B2 and their major hydrolysis products in corn, feed, and meat, using HPLC. *J. Agric. Food Chem.* **1996**, *44*, 1047−1052.

Thiel, P. G.; Marasas, W. F. O.; Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A. The implication of naturally occurring levels of fumonisins in corn for human and animals health. *Mycopathologa* **1992**, *119*, 3−9.

Trucksess, M. W. Mycotoxins. *J. AOAC Int.* **1998**, *81*, 128−137.

Usleber, E.; Straka, M.; Terplan, G. Enzyme immunoassay for fumonisin B1 applied to corn-based food. *J. Agric. Food Chem.* **1994**, *42*, 1392−1396.

Ware, G. M.; Francis, O.; Kuan, S. S.; Umrigar, P.; Carman, A.; Carter. L.; Bennett, G. A. Determination of fumonisin B1 in corn by high performance liquid chromatography with fluorescence detection. *Anal. Lett.* **1993**, *26*, 1751−1760.

Wilson, D. M.; Sydenham, E. W.; Lombaert, G. A.; Trucksess, M. W.; Abramson, D.; Bennett, G. A. Mycotoxin analytical techniques. In *Mycotoxins in Agriculture and Food Safety*; Sinha, K. K., Bhatnagar, D., Eds.; Dekker: New York, 1998; pp 135−182.

Yeung, J. M.; Prelusky, D. B.; Savard, M. E.; Dang, B. D. M.; Robinson, L. A. Sensitive immunoassay for fumonsisin B1 in corn. *J. Agric. Food Chem.* **1996**, *44*, 3582−3586.

Yu, F. Y. Immunochemical studies on fumonisins. Ph.D. dissertation, University of Wisconsin−Madison, Madison, WI, 1997.

Yu, F. Y.; Chu, F. S. Production and characterization of antibodies against fumonisin B1. *J. Food Prot.* **1996**, *59*, 992−997.

Zhou, H. R.; Pestka, J. J.; Hart, L. P. Molecular cloning and expression of recombinant phage antibody against fumonisin B-1. *J. Food Prot.* **1996**, *59*, 1208−1212.

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