Production and Characterization of a Monoclonal Antibody to Aflatoxin B_2

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Aflatoxin B_2–oximinoacetate (AFB_2–OA), a protein-reactive derivative of aflatoxin B_2 (AFB_2), was bound to ethylenediamine-modified BSA (EDA–BSA) and the resulting AFB_2–EDA–BSA conjugate was used to immunize BALB/c mice. Hybridomas were prepared from mice, which were demonstrated to produce anti-AFB_2 antibodies by indirect enzyme-linked immunosorbent assay (ELISA). Three monoclonal antibody-producing hybridomas were isolated. One monoclonal antibody reacted with an EDA–human serum albumin epitope. A second monoclonal antibody was partially reactive with AFB_2. A third monoclonal antibody, named 4H2, was very specific for AFB_2, exhibiting at most 19% cross-reactivity with any naturally occurring aflatoxin. In the competitive indirect ELISA, as little as 50 pg of AFB_2 could be detected utilizing the 4H2 monoclonal antibody. This monoclonal antibody based immunoassay seems well suited to screen suspect food samples for aflatoxin contamination.

Consumption of food contaminated with aflatoxins, metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, has been linked to human primary hepatocellular carcinoma (Linsell and Peers, 1977), as well as other human and animal diseases (Campbell and Stoloff, 1974). Aflatoxin B_2 (AFB_2) is a biosynthetic derivative of the more toxic parent compound aflatoxin B_1 (AFB_1). Although in rare instances AFB_2 has been reported to be produced by fungal isolates that did not produce AFB_1 (Schroeder and Carlton, 1973), these aflatoxins normally occur together.

Prevention of exposure to aflatoxins depends upon rapid detection of contaminated food commodities and removal of these contaminated commodities from the market (Linsell, 1979). Potentially the most useful and rapid methods for detecting aflatoxins are the various immunoassays that have been described in the past few years (Chu, 1984). Since aflatoxins by themselves are too small to be immunogenic, production of anti-aflatoxin antibodies for use in immunoassays depends on the ability to produce aflatoxin conjugates with protein that are immunogenic. The various methods for producing aflatoxin–protein conjugates have been reviewed elsewhere (Hastings et al., submitted). Antibodies to hapten-carrier conjugates generally recognize that part of the hapten distal to the point of attachment to the protein carrier (Hammock and Mumma, 1980); thus, selection of the method for producing aflatoxin hapten depends upon the desired specificity of the antibody. Langone and Van Vunakis (1976) described the production of antibodies to aflatoxin B_1 by attaching the aflatoxin to polylysine using a (carboxymethyl)oxime bridge at the cyclopentenone moiety. The resulting rabbit antibodies thus recognized specifically the 2,3-double-bond region of aflatoxin B_1.

Highly specific monoclonal antibodies have been developed to aflatoxin B_1 (Blankford and Doerr, 1986; Sun et al., 1983), aflatoxin B_1–DNA adducts (Haugen et al., 1981; Hertzog et al., 1982), and aflatoxin M_1 (Woychik et al., 1984), for use in immunoassays. The advantages of using monoclonal antibodies rather than heterologous antibodies include the ability to produce virtually unlimited amounts of homogeneous and highly specific antibodies utilizing a relative impure antigen preparation (Kohler and Milstein, 1975; Scharff et al., 1981). It is difficult to obtain monoclonal antibodies to weak immunogens, however (Scharff et al., 1981). In the case of hapten-carrier conjugates, an important factor in determining the level of the immune response is the molar ratio of the hapten to the carrier (Hammock and Mumma, 1980). Low molar ratios will generally produce weak immunogens. Chu et al. (1982) reported that ethylenediamine modification of protein carriers increased their efficiency for conjugation of mycotoxin derivatives.

In this study, monoclonal antibody-producing hybridomas were produced from mice that were immunized with an aflatoxin B_2–oximinoacetate–ethylenediamine–bovine serum albumin (AFB_2–EDA–BSA) conjugate. This conjugate was selected since it was believed to be necessary to bridge the antigenic molecule of interest (AFB_2) away from the protein carrier to obtain a more specific antibody. The reactivity of the monoclonal antibodies produced were characterized by indirect enzyme-linked immunosorbent assay (indirect ELISA) and their specificities for AFB_2 determined by competitive indirect ELISA. One monoclonal antibody was isolated that was highly specific for AFB_2. The sensitivity and specificity of the competitive indirect ELISA using this monoclonal antibody was demonstrated. The enhanced effect of EDA modification of carrier was discussed. This monoclonal antibody based method seems applicable for the large-scale routine screening of food samples contaminated with aflatoxin because of its sensitivity and ability to be semiautomated.

MATERIALS AND METHODS

Conjugate Preparation. Aflatoxin B_2–oximinoacetate (AFB_2–OA) was prepared as previously described (Hastings et al., submitted). Aflatoxin B_1–oximinoacetate (AFB_1–OA) was prepared as described by Chu et al. (1977). AFB_1–OA and AFB_2–OA were crystallized by an adaptation of the method of Thouvenot and Morfin (1983) as previously reported (Hastings et al., submitted).

EDA-modified bovine serum albumin (EDA–BSA), guinea pig serum albumin (EDA–GSA), and human serum albumin (EDA–HSA) were gifts from Dr. Bradford Brooks, IBM Corp., Boulder, CO. The EDA to albumin ratios were reported to be 28:1 for EDA–BSA, 19:1 for EDA–GSA, and 27:1 for EDA–HSA. AFB_2–carrier conjugates were pre-

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pared according to the method of Chu and Ueno (1977). Twenty milligrams of EDA-modified albumin, 10 mg of AFB_2–OA, and 750 mg of 1-ethyl-3,3-[(dimethylamino)-propyl]carbodiimide (EDPC, Sigma Chemical Co., St. Louis, MO) were added sequentially to 20 mL of 25% ethanol. The solution was then stirred for 48 h at room temperature with addition of two 750-mg portions of EDPC. After 48 h, the solution was transferred to dialysis tubing (molecular weight cutoff 12 000–14 000; Spectrum Medical Industries, Los Angeles, CA) and suspended in 1 L of Dulbecco's phosphate-buffered saline (Gibco Laboratories, Grand Island, NY). The solution was dialyzed at 4 °C for 3 days with five changes of PBS. After dialysis, the conjugate was filter sterilized (Acrodisc filter, 0.2-μm pore size, Gelman Filtration Products, Ann Arbor, MI) and stored in 1-mL aliquots at −70 °C.

The protein concentration of each conjugate was determined spectrophotometrically by the method of Bradford (1976) (Coomassie blue in methanol and phosphoric acid; Quan T test, Quantimetrix, Hawthorne, CA) using a COBAS BIO analyzer (Roche Analytical Instruments, Nutley, NJ). The concentration of AFB_2–OA hapten was determined spectrophotometrically at 363 nn on a Gilford 300N spectrophotometer (Gilford, Oberlin, OH).

Preparation of Hybridomas. Twelve female BALB/c mice, 6 weeks old, were obtained from Charles River Breeding Laboratories (Cambridge, MA). A 1:2 emulsion of AFB_2–EDA–BSA in Freund's complete adjuvant (Gibco Laboratories) was prepared, and the mice were immunized by intraperitoneal injection of 0.2 mL (22 μg of AFB_2–EDA–BSA) each. The mice were given three booster immunizations by subcutaneous injection of a 1:2 emulsion of AFB_2–EDA–BSA in Freund's incomplete adjuvant (Gibco) at 1-month intervals, 0.2 mL/injection.

Serum was obtained from the mice by retroorbital bleeding and tested for specific antibody by indirect ELISA (described below.) Five days prior to fusion, two mice producing antibodies reactive in the indirect ELISA were boosted by tail vein injection of 66 μg of AFB_2–EDA–BSA in 0.2 mL of PBS.

The mouse myeloma cell line P3X63-Ag8.653 (a gift from Dr. G. Yancey Gillespie, University of North Carolina at Chapel Hill) was maintained at 37 °C in 5% CO_2 atmosphere in a 1:1 mixture of 4.5 g/L glucose Dulbecco's modified Eagle's medium (DMEM): Ham's F-12 nutrient medium (Gibco Laboratories) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 1 mM/mL sodium pyruvate (Gibco), 50 μg/mL gentamicin sulfate (KB Biologicals, Lenexa, KA), 2.5×10^{-4} mM/mL HEPES buffer (Gibco), 2 mM/mL L-glutamine (Sigma), and 20 μg/mL 8-azaguanine (Sigma). The day prior to hybridization, the myeloma cells were transferred to 10% FBS 1:1 DMEM–Ham's F-12 without 8-azaguanine and incubated overnight under the above conditions. On the day of hybridization, the myeloma cells were collected by centrifugation at 600*g*, washed twice in Hank's balanced salt solution with 50 μg/mL gentamicin sulfate (HBSS, Flow Laboratories), and suspended in 1:1 DMEM–Ham's F-12 without FBS.

The mice were killed by decapitation and their spleens removed and placed in ice-cold HBSS. The spleens were washed twice in HBSS and then transferred to Falcon 3003 sterile plastic Petri dishes (Becton-Dickinson, Paramus, NJ). The spleen cells were removed by injecting each spleen with 10 mL of serum-free 1:1 DMEM–Ham's F-12 using a 23-guage needle bent at a 45° angle. The cells were collected in 50-mL sterile conical tubes containing serum-less culture medium and kept on ice. Large debris was allowed to settle out for 5 min; the suspended cells were transferred to sterile conical tubes and collected by centrifugation. Red blood cell lysing buffer (a sterile solution of ammonium chloride, potassium carbonate, and ethylenediaminetetraacetic acid; Sigma) was added to the splenocytes and kept on ice for 5 min. The cells were then washed twice in serum-free culture medium.

Prior to fusion P3X cells and splenocytes were counted on a Coulter ZM (Coulter Electronics, Hialeah, FL), and percent viability was determined microscopically by the trypan blue dye exclusion method. Splenocytes (1.5×10^8) and myeloma cells (5×10^7) were combined in a sterile conical tube and centrifuged for 10 min at 600*g*. The medium was aspirated off, and the cell mixture was agitated to break up clumps.

Poly(ethylene glycol) (PEG, MW 1500; Mallinckrodt Chemical Co., St. Louis, MO) was autoclaved, mixed 1:1 with serumless DMEM, and kept at 37 °C in a 5% CO_2 incubator. The PEG solution (0.8 mL) was added to the splenocyte–myeloma cell mixture over a period of 1 min with mixing. The resulting mixture was kept at 37 °C for 1 min, and 21 mL of serum-free DMEM–Ham's F-12 was added. The cells were collected by centrifugation and suspended in 20 mL of warm HAT medium, consisting of 1:1 DMEM–Ham's F-12 supplemented with 10% FBS, sodium pyruvate, HEPES buffer, gentamicin sulfate, L-glutamine, 30 μg/mL endothelial cell growth supplement (ECGS, Collaborative Research, Inc., Lexington, MA), 13.61 μg/mL hypoxanthine (Sigma), 3.88 μg/mL thymidine (Sigma), 0.22 μg/mL glycine (Sigma), and 0.176 μg/mL aminopterin (Sigma). The cell mixture was then placed in a tissue culture dish (Falcon 3003) and maintained overnight at 37 °C in a humidified 5% CO_2 incubator.

The cells were then collected and distributed at a density of about 2×10^5 viable cells per well in 96-well sterile culture trays (3596; Costar, Cambridge, MA). Fresh HAT medium was added at 7 days, and the plates were examined for growth after 14 days. Wells positive for growth were assayed for antibody production by an indirect ELISA method (described below.)

Wells testing positive for antibody production by indirect ELISA were expanded by a modified limiting dilution technique. HT medium, composed of 1:1 DMEM–Ham's F-12 with 10% FBS, ECGS, gentamicin sulfate, HEPES buffer, L-glutamine, sodium pyruvate, hypoxanthine, thymidine, and glycine, was added to 96-well sterile microculture plates (100 μL/well) except for the last column of wells into which 50 μL of medium was added. Cells were transferred from positive wells to the first well in the first column of the expansion plates. The cells were serially diluting down the first column and then across each row by transferring 50 μL from each well to the next. The plates were examined for growth after 7–10 days. Wells positive for growth were assayed for antibody production by the indirect ELISA method.

Wells positive for antibody to AFB_2–EDA–G were then expanded by transferring to 24-well sterile cell culture plates (3599; Costar) and subsequently expanded to 6-well plates (3506; Costar), 50-mL flasks (Intermed Nunclon), and 500-mL cell culture flasks (Costar). Cells in logarithmic growth were frozen by control rate freezing in a 7:2:1 mixture of HT media–FBS–DMSO and stored in the vapor phase of a liquid nitrogen freezer.

ELISA Methods. An indirect ELISA was developed to detect and quantitate anti-AFB_2 antibody production in mice. AFB_2–EDA–GSA, 10 μg in 50 μL of carbonate-bicarbonate buffer/well, was adsorbed to 96-well poly-

(ethylene terephthalate glycol) (PETG) microtiter plates (Costar, Cambridge, MA) by overnight incubation at 4 °C. The plates were washed three times with 0.05% Tween 20 (Fisher Scientific Co., Springfield, NJ) in PBS (PBS–Tween) and blocked by incubating for 1 h at 37 °C with 200 μL/well 0.25% ovalbumin (Sigma) in PBS–Tween (ovalbumin–PBS–Tween). After the plates were washed three times with PBS–Tween, serial dilutions of mouse sera in ovalbumin–PBS–Tween were added to the plate followed by overnight incubation at 4 °C. The microtiter plates were washed three times with PBS–Tween, and goat antimouse Ig immunoglobulin alkaline phosphatase conjugate (Bionetics Laboratory Products, Charleston, SC; diluted 1:500 in ovalbumin–PBS–Tween) was added, 50 μL/well followed by incubation for 1 h at 37 °C. The plates were developed by adding 1 mg/mL *p*-nitrophenyl phosphate (Sigma) in carbonate–bicarbonate buffer, 100 μL/per well, incubation at 37 °C, and reading at λ 405 nm on a Titertek Multiskan (Flow Laboratories, McLean, VA). The well containing the highest dilution of mouse antiserum giving an absorbance greater than twice the standard deviation of the mean of background absorbance was taken as the titer of the antiserum.

This indirect ELISA was used to screen hybridomas for anti-AFB_2 antibody production. After adsorption of AFB_2–EDA–GSA to PETG microtiter plates, washing with PBS–Tween, and blocking with ovalbumin–PBS–Tween, 50 μL of culture fluid was added to each well followed by overnight incubation at 4 °C. Mouse antiserum positive for antibody to AFB_2 was used as a positive control (diluted 1:1000 in ovalbumin–PBS–Tween.) The plates were washed, and 50 μL/well goat antimouse Ig immunoglobulin–alkaline phosphatase conjugate (1:500 in ovalbumin–PBS–Tween) was added. Following incubation at 37 °C for 1 h, the plates were washed, *p*-nitrophenyl phosphate solution was added, and the plates were developed at 37 °C and read at λ 405 nm.

A competitive indirect ELISA was used to characterize the reactivity of the antibodies. AFB_2–EDA–HSA, 10 μg in 100 μL of buffer/well, was adsorbed to a PETG plate by overnight incubation at 4 °C. AFB_2 standards were prepared by dissolving AFB_2 in DMSO (100 μg of AFB_2/mL of DMSO) with subsequent dilution in PBS–Tween, yielding standards with the following concentrations: 10000, 5000, 1000, 500, 100, 50, 10, 5, 1, and 0 ng/mL (1:100 DMSO–PBS–Tween). After the plates were washed and blocked with ovalbumin–PBS–Tween, standards were added, 50 μL/well, followed by addition of 50 μL/well of cell culture supernatant. The plates were incubated overnight at 4 °C and washed, and 100 μL/well of goat antimouse Ig immunoglobulin–alkaline phosphatase conjugate (1:500 in ovalbumin–PBS–Tween) was added. After incubation and washing, *p*-nitrophenyl phosphate solution was added, and the plates were developed at 37 °C and read at λ 405 nm.

The cross-reactivities of antibodies from hybridoma 4H2 were tested in the competitive indirect ELISA using AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, AFB_{2a}, and AFG_{2a} standards (Sigma, prepared as described above,) and crystallized AFB_1–OA and AFB_2–OA. AFB_2–EDA–HSA, 10 μg in 100 μL of carbonate–bicarbonate buffer per well, was adsorbed to PETG plates by overnight incubation at 4 °C. The plates were washed, blocked with ovalbumin–PBS–Tween, and washed again. Aflatoxin standards were added to the plate, 50 μL/well, followed by addition of 50 μL of cell culture supernatant. The plate was incubated overnight at 4 °C and washed and goat anti-mouse Ig immunoglobulin–peroxidase (1:100 in ovalbumin–PBS–Tween) added. After being incubated for 1 h at 37 °C and washed, the plate was developed by addition of 100 μL of *O*-phenylenediamine (Sigma) in citrate–phosphate buffer. Following incubation at room temperature, the plates were read on a Titertek Multiskan, using a λ 450 nm filter.

The isotype of monoclonal antibody 4H2 was determined by modification of the indirect ELISA. AFB_2–EDA–HSA was adsorbed to a PETG microtiter plate as described above. The plate was washed, and serial dilutions of cell culture supernatant in ovalbumin–PBS–Tween were added. After overnight incubation at 4 °C and washing with PBS–Tween, 1:100 dilutions in ovalbumin–PBS–Tween of the following peroxidase-conjugated antisera were added to the plate: rabbit antimouse antisera to IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, 7sIg, IgM, κ light chain, Ig immunoglobulin (Bionetics Laboratory Products). The plate was incubated for 1 h at 37 °C, washed, incubated at room temperature after addition of OPD, and read at λ 450 nm.

RESULTS

The ratio of hapten (AFB_2–OA) to carrier (EDA–albumin) was calculated for each conjugate preparation based on the assayed protein concentration and the calculated total absorbance of the hapten at 363 nm. The absorbance was determined for the conjugate diluted in carbonate–bicarbonate buffer, and the spectrophotometer was set at zero against a blanking solution containing the carrier albumin at the approximate concentration of the carrier–protein complex. The molecular weight of albumin was assumed to be 68000, and the molar absorptivity of AFB_2–OA was reported to be 22060 (Hastings et al., submitted). The calculated molar ratios of AFB_2–OA to EDA–albumins were 27.5 for AFB_2–EDA–BSA, 25.4 for AFB_2–EDA–GSA, and 85.0 for AFB_2–EDA–HSA.

By indirect ELISA, the anti-AFB_2 titers in the mice used to generate hybridomas were greater than 1:10240. Mice with anti-AFB_2 titers lower than 1:10240 did not yield stable clones producing anti-AFB_2 antibody.

Three monoclonal antibodies (Moabs), named 3A3, 4H2, and 5B7, were characterized for their reactivity with AFB_2. Moab 3A3, although reactive with AFB_2–EDA–GSA, was found upon further analysis to be specific for an EDA–HSA epitope. Binding of Moab 3A3 to AFB_2–EDA–HSA in the competitive indirect ELISA was not blocked by addition of AFB_2, but Moab 3A3 did bind to EDA–HSA in the indirect ELISA. Moab 5B7 was partially reactive with AFB_2 in the competitive indirect ELISA, but even with the 1 μg/mL AFB_2 standard (25 ng total AFB_2) complete reactivity (total blocking of antibody binding to competitive ligand) was not observed, indicating that the antibody was not completely specific for AFB_2. Antibodies from clone 5B7 did not appear to be reactive with EDA-modified protein.

Moab 4H2, determined to be an IgG_1, κ light-chain isotype by indirect ELISA, was found to be specifically reactive with AFB_2. The results of competitive indirect ELISA studies with Moab 4H2 are shown in Figures 1 and 2. As seen in Table I, the only naturally occurring aflatoxin examined that demonstrated any appreciable cross-reactivity with Moab 4H2 was AFB_1, which differs from AFB_2 by two protons. The concentration of AFB_1 necessary to produce 50% inhibition of Moab 4H2 binding to AFB_2–EDA–HSA was 10 times greater than the amount of AFB_2 needed to produce 50% inhibition of binding. The calculated cross-reactivity of AFB_1 with Moab 4H2 was 19%. The reactivities of Moab 4H2 for AFB_2–OA and AFB_1–OA were only 49% and 50%, respectively, of the reactivity with AFB_2. At the highest standard concen-

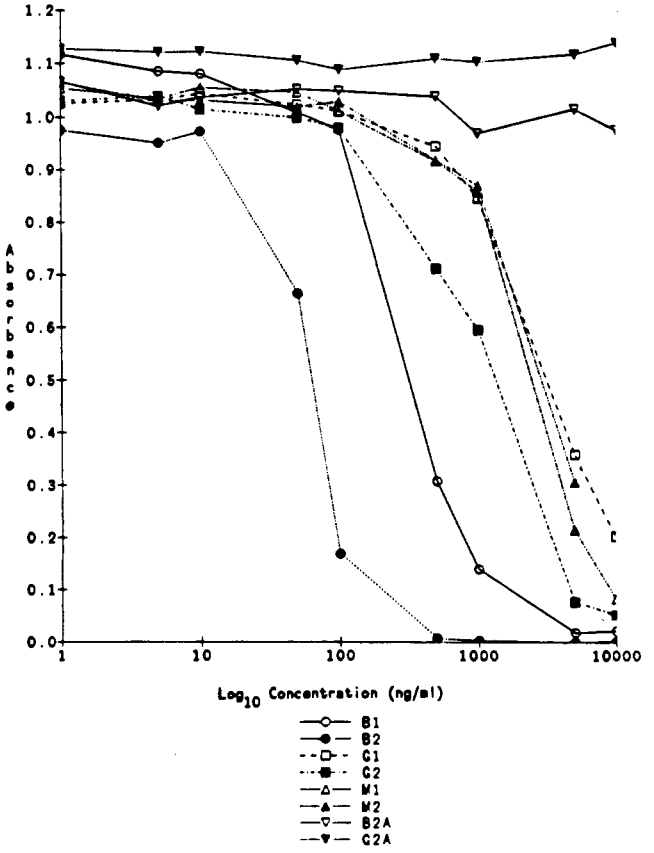

Figure 1. Results of the competitive indirect ELISA using Moab 4H2 and AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, AFB_{2a}, and AFG_{2a} standards.

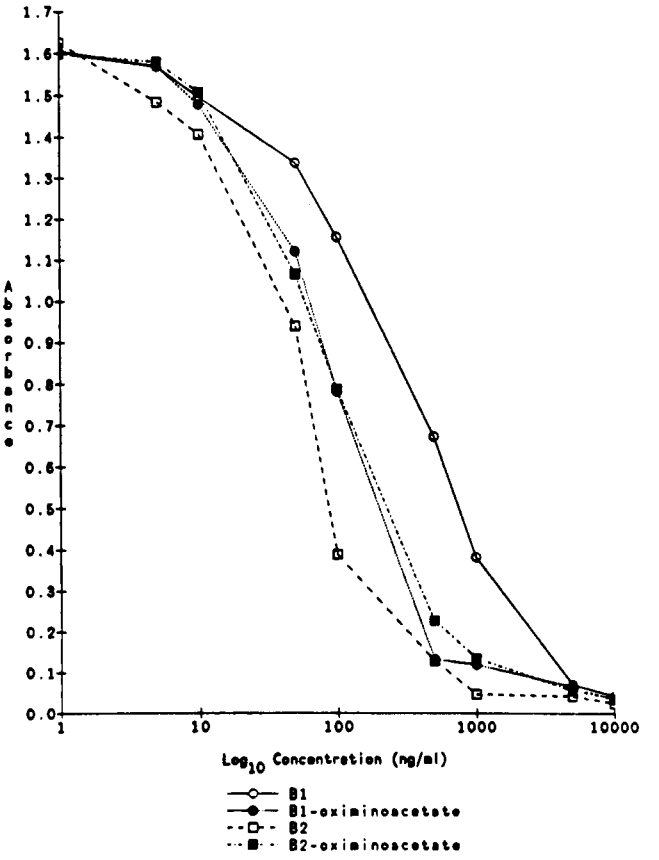

Figure 2. Results of the competitive indirect ELISA using Moab 4H2 and AFB_1, AFB_2, AFB_1–OA, and AFB_2–OA standards.

Table I. Evaluation of the Competitive Indirect ELISA Using Moab 4H2

aflatoxin std	50% inhib concn[a]	% cross-react.[b]	aflatoxin std	50% inhib concn[a]	% cross-react.[b]
AFB_2	50	100	AFM_1	5000	<1
AFB_2–OA	100	49	AFM_2	5000	<1
AFB_1	500	19	AFB_{2a}	>10000	<1
AFB_1–OA	100	50	AFG_{2a}	>10000	<1
AFG_1	5000	<1			
AFG_2	1000	<1			

[a] Mean corrected absorbance of 0 ng/mL standard (1:100 DMSO in PBS–Tween) at λ 450 nm = 1.348. Concentration of aflatoxin standard (ng/mL) giving 50% inhibition of antibody binding to the hapten–carrier conjugate. [b] The cross-reactivity of Moab 4H2 calculated as the ratio of absorbances of the aflatoxin standards at 50 ng/mL, the 50% inhibitory concentration of AFB_2.

tration (10 μg/mL), only AFB_2 completely blocked binding of Moab 4H2 to AFB_2–EDA–HSA, indicating that the specificity of the monoclonal antibody persisted over a fourfold range of standard concentrations. The absorbance of the 10 ng/mL AFB_2 standard was only 91% of the zero standard absorbance, indicating that, in the competitive indirect ELISA, Moab 4H2 could detect as little as 50 pg of AFB_2 (since 50 μL of standard was assayed) with virtually no cross-reactivity with AFB_1.

DISCUSSION

Development of immunoassays for detection and quantitation of toxic chemicals depends on the ability to produce highly class- or molecule-specific antibodies to small molecular weight compounds (haptens) by binding them to immunogenic carrier proteins. These immunoassays have the advantage of sensitivity, speed, and low cost for screening mixtures for the molecular contaminant of interest. Disadvantages of immunoassays utilizing conventional antisera are the requirements for a continuous source of immunized animals and significant cross-reactivity with structurally similar compounds. This cross-reactivity can be difficult to predict and can vary significantly with different lots of heterologous antisera. In contrast, development of hybridoma technology has provided a means for continuous production of monospecific antibodies of known biological activity and invariant specificity.

Several parameters determine the changes of successfully producing a monoclonal antibody to a hapten. Although weak antigens can be used to produce monoclonal antibodies, chances of success improve with antigens that produce stronger immune responses. To induce a strong immune response to a chemical hapten, the hapten to carrier molar ratio should be sufficiently great to induce an immune response that is directed more to the hapten than the carrier alone. Use of ethylenediamine modification of carrier provides two important advantages: It increases the number of potential binding targets on the carrier molecule, and it introduces a spacer between the carrier and the hapten (Chu et al., 1982) that should enhance the recognition of the hapten by antigen reactive lymphocytes. The second effect improves the specificity of resulting antibodies by rendering the hapten more immunogenic and by reducing steric hindrance of antibody binding by the carrier.

Purity of antigen is a less important consideration in producing monoclonal antibodies to hapten (Scharff et al., 1981) than with heterologous antibody production. Indeed, relatively impure antigens can have an adjuvant-like effect on antigen potency, although increasing the likely cross-reactivity of the resulting antisera (Hurn and Chantler, 1980). Thus, when a mouse is immunized to obtain hapten-specific monoclonal antibodies, one can take advantage

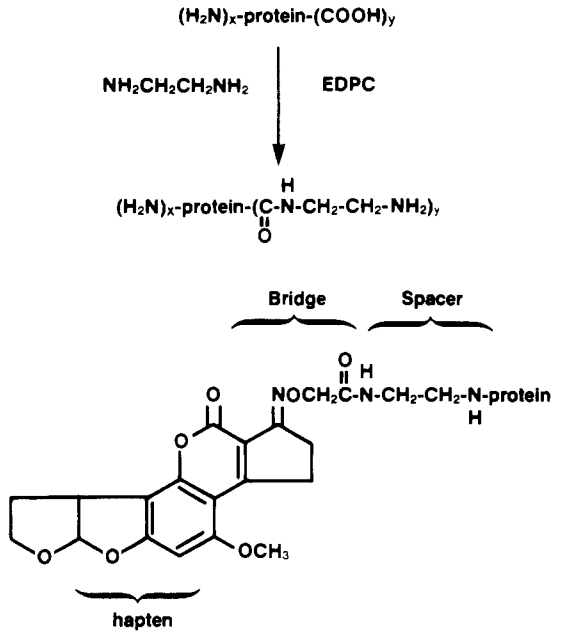

Figure 3. Top: Reaction of ethylenediamine (EDA) with protein in the presence of carbodiimide results in two structurally different hapten binding sites, one with an EDA spacer (the right-hand side of the product molecule) and one without a spacer (the left-hand side of the product molecule) (Chu et al., 1982). Bottom: Structure of AFB_2–EDA–protein.

of this immune enhancing effect of impure antigens and still obtain very specific monoclonal antibodies through clonal selecting and screening.

The AFB_2–EDA–BSA conjugate is antigenically very complex. The hapten can be bound to the carrier in two forms: attached directly to EDA or attached to ϵ-amino functions on lysine (Figure 3). With carbodiimide as the coupling agent, urea haptens can be formed as byproducts (Goodfriend et al., 1964). AFB_2–OA, when prepared as described elsewhere (Hastings et al., submitted), results in a product that is approximately 90% pure. With use of crude reaction product to prepare the hapten–carrier conjugate, as was done in this study, there is also the possibility that unintended hapten(s) was also attached to the immunogen.

In summary: (1) Very specific monoclonal antibodies were obtained with use of a semipure hapten–carrier preparation. (2) The ethylenediamine spacer appears to have improved the specificity of the monoclonal antibody for AFB_2. (3) The ELISA method developed with anti-aflatoxin B_2 monoclonal antibodies was both sensitive (detecting as little as 50 pg of AFB_2) and specific (exhibiting only up to 19% cross-reactivity with the other naturally occurring aflatoxins tested).

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