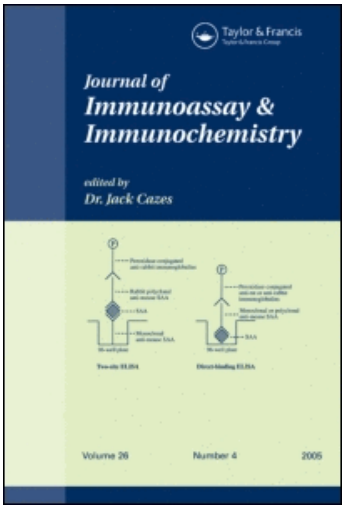

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Production and Characterization of Monoclonal Antibodies Specific to Atrazine Group Compounds: Effects of Coating Ligand Structure on the Variation of Sensitivity and Specificity

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC TO ATRAZINE GROUP COMPOUNDS: EFFECTS OF COATING LIGAND STRUCTURE ON THE VARIATION OF SENSITIVITY AND SPECIFICITY

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Bioanalysis & Biotransformation Research Center[1], Korea Institute of Science & Technology, P. O. Box 131 Cheongryang, Seoul 130-650, College of Pharmacy[2], Seoul National University, Seoul 151-742, Sogang University[3], Seoul 121-742, Korea

ABSTRACT

Hybridoma cells were prepared by immunizing mice with carboxylic derivatives of atrazine conjugate to bovine serum albumin. After the screening of culture supernatant of hybridomas, five cell lines producing monoclonal antibodies were established and 1.8–5.3 ml of ascitic fluid per mouse was obtained from each cell line. The protein A affinity purification yielded 0.35 – 0.65 mg per ml of ascitic fluid from each cell line. The characterization studies in terms of sensitivitiy and specificity indicate that MAb 2F9 and MAb 4B9 showed the best responses with atrazine and its group of ametryne and cyanazine, using microtiter plate coated with simazine derivative of 6-amino hexanoic acid; no cross-reactivity was shown with simazine and cyanuric chloride. (KEY WORDS: atrazine, simazine, monoclonal antibody, purification, sensitivity, specificity)

INTRODUCTION

Atrazine and its derivatives, the family of triazines, are selective herbicides for the control of annual glasses and broad-leaved weeds. As a

result of popular use during the 1980s, the residues may have contaminated ground water, streams, soil, and crops. These herbicides are very toxic to animals and have been classified as possible human carcinogens. Their toxicities are a warning to people and their residues are monitored in water and foods (1-4). Most of the detecting systems have been liquid or gas chromatography methods (5) which are not adequate to use outside the laboratory, due to time-consuming sample preparation and delicate instrumentation steps. Recent development of an immunotest applying the antigen-antibody reaction is very popular for the screening of environmental contaminants out of a large volume of samples on-site, mainly due to their specificity and simplicity.

In this paper, atrazine and simazine were derivatized to form carboxylic derivatives and conjugated with carrier proteins by the carbodiimide method (6) for use as immunogens. Monoclonal antibodies using the atrazine-BSA (1b-BSA) immunogen were prepared and characterized to select the proper antibodies for use in an immunoassay. Titers were measured by the indirect ELISA method. After protein A purification, the binding characteristics and the cross-reactivity were compared using the indirect competitive ELISA method.

MATERIALS AND METHODS

Materials

Carboxylic derivatives of simazine and atrazine were prepared as in Goodrow (6). Ametryne and simetryne were purchased from Aldrich

(Milwaukee, WI.), and cyanazine and trifluralin were purchased from Dr. Ehrenstorfer (GmbH). Bovine serum albumin (BSA), N-hydroxysuccin-imide, 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide HCl (EDC), anti-mouse HRP-conjugated IgG, Freunds complete adjuvant (FCA), Freunds incomplete adjuvants (FIC), and *o*-phenylenediamine were purchased from Sigma Chemical Co. (MO.). In addition, the following reagents were used: keyholelimpet hemocyanin (KLH, Pierce, IL.), dicyclohexylcarbodiimide (Aldrich, WI., USA), rabbit anti-sheep IgG (Cappel, NC), and microtiter plate well (maxisorp, Nunc, Denmark). Eight-week old female mice (Balb/c) were used for producing ascitic fluids. An ELISA reader (Molecular Devices, CA) was used to measure the absorbances at 490 nm.

Preparation of immunogen and coating ligands

Simazine and atrazine were derivatized to a carboxyl group according to the method of Goodrow (6). Figure 1 shows the carboxyl derivatives of simazine (1a), atrazine (1b), and cyanuric chloride (1c, 1d, 2a, 2b, 2c, and 2d), where BSA was conjugated for immunogen use or KLH was conjugated for coating ligands. The conjugation steps consisted of carboxylic ligand activation and carrier protein conjugation by the modified method of Yoon (7). 0.2 mmole of carboxylic derivatives of simazine or atrazine in 0.5 ml dimethylforamind (DMF) was mixed for 4 hours at room temperature with 0.2 mmole solid n-hydroxysuccinimde (NHS) and 0.22 mmole solid

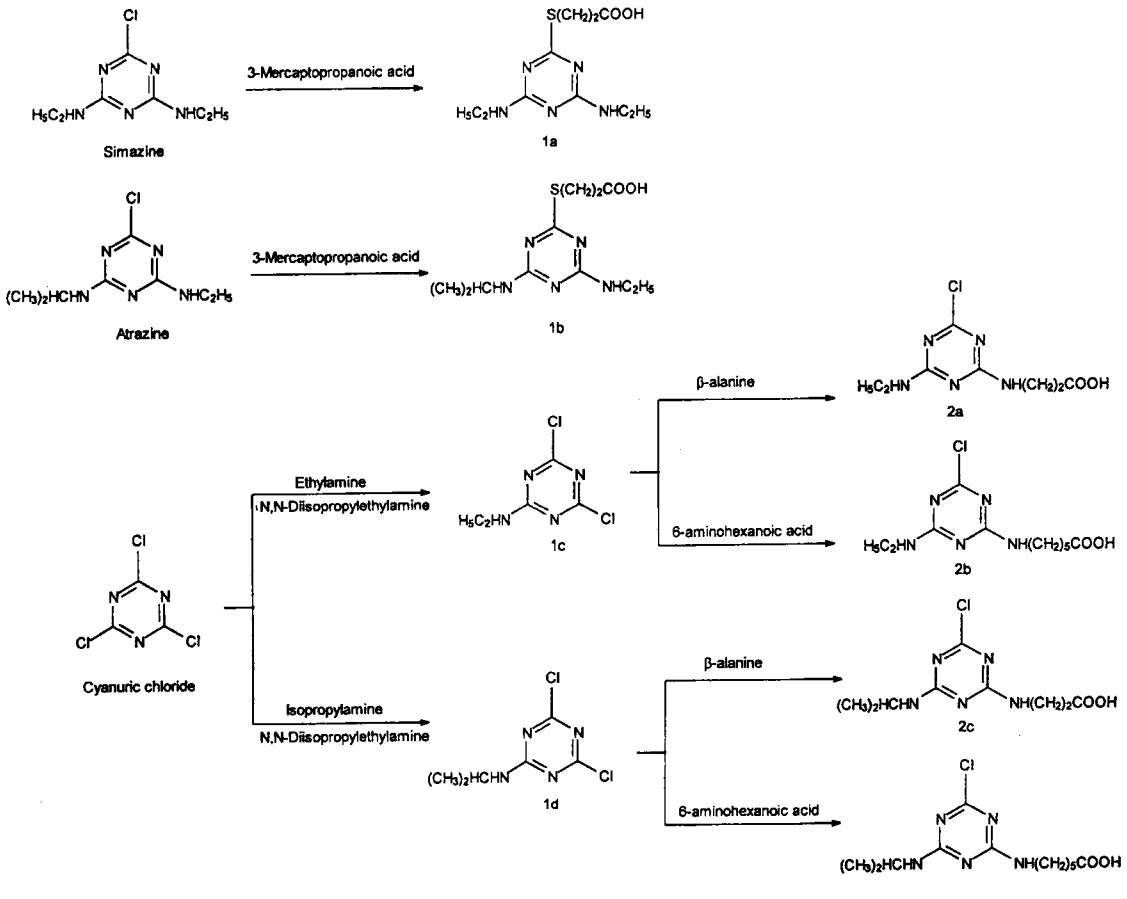


FIGURE 1. The chemical structure of the derivatized carboxylic ligand of triazines.

dicyclohexylcarbodiimide (DCC) used for the activation. The resulting by-product of dicyclohexyl urea was removed by centrifugation. The NHS-activated ligand was conjugated with BSA (10 mg/ml) or KLH (10 mg/ml) by adding fractions of 0.1 mmol of ligand, dropwise. The mixture was stirred slowly and the formed precipitate was dissolved adding DMF dropwise, keeping the pH of the mixture between 10 and 11. The mixture was reacted for 22 hours at 4°C. The ligand-protein conjugate was dialyzed against 1 mM PBS and protein concentrations were measured using absorbances at 280 mm.

Preparation of hybridoma clones and ascitic fluids specific to atrazine

Atrazine-BSA conjugate (molar ratio of atrazine to BSA for the preparation, 200:1) was used as an immunogen. Spleen cells of Balb/c mice immunized with the immunogen were fused with a mouse myeloma cell line, p3-x63-Ag 8.653, in the presence of 45% polyethylene glycol-5% dimethylsulfoxide using the hybridoma technique described in Yoon (7). The culture supernatants of the hybridoma cells were screened by a binding assay of atrazine using six kinds of atrazine-KLH coated microtiter plate (coating; 1μg/ml in 10 mM PBS containing 0.05% tween-20, 200 μl/well) by the indirect competitive ELISA method. The well was post-coated using 3% BSA. An aliquot of 100 μl of hybridoma supernatant and 100 μl of various concentrations of atrazine (0, 0.01, 0.1, 1.0, 10 μg/ml) were incubated in the

well for 2 hours at RT. After wash 3 times with 10 mM PBST, 200 μl antimouse IgG-HRP conjugate (1:2,000 diluted with PBS containing 1% BSA) were incubated for 2 hours at RT. After wash 3 times with 10 mM PBST, 200 μl OPD substrate were added and incubated for 10 min. at RT. After quenching with 50 μl 4N-H_2SO_4, the absorbance was read at 490 nm using ELISA reader (Molecular Devices, CA).

The hybridoma cells that showed marked atrazine binding activity were cloned, at least twice, by the method of limiting dilutions. Five hybridoma cell lines producing monoclonal antibodies against atrazine (anti-1b-BSA) were established and ascitic fluid were prepared by innoculating cell lines to Balb/c mouse primed with pristane.

Purification of monoclonal antibody by protein A affinity chromatography

Monoclonal antibodies were purified from the ascitic fluid. Protein A gel (Amershampharmacia Biotech, Uppsala, Sweden) was washed with water to remove ethanol. The gel was packed into a column and prewashed with wash buffer (50 mM tris buffer, pH 8.0). Ascitic fluid, diluted with a volume of wash buffer and filtered through a 0.45 μm filter, was applied into the column. After 2 hours incubation at RT, the unbound proteins were washed off with wash buffer until no protein was detected in the effluent. The antibody was eluted using 0.1 M glycine HCl buffer, pH 3.0 and 0.5 ml fractions were

collected in tubes containing 15 µl of 2 M trisma. The specific antibody fractions were pooled and dialyzed against 10 mM PBS and concentrated to 1.0 mg/ml using an Amicon concentrator.

Titration of antibody level by indirect competitive ELISA

The antibody levels were measured by the indirect competitive ELISA method as described in the previous report (8). Briefly, 200 µl of KLH-conjugated simazine or KLH-conjugated atrazine in 50 mM carbonate buffer, pH 9.6, was coated on microtiter plate overnight at 4°C and 200 µl of 3 % BSA was post-coated for 2 hours at RT. After washing, serially diluted specific antibody were incubated in the well for 2 hours at RT. After washing the unbound antibodies with 10 mM PBST three times, 200 µl of diluted anti-mouse IgG-HRP conjugate was incubated for 2 hours at RT. After washing three times with PBST, 200 µl OPD substrate was added and incubated for 10 minutes. 50 µl of 2N H_2SO_4 was added to stop the color reaction. The absorbance was measured at 490 nm. The titer level was determined by the MAb concentration showing absorbance 2.0 at 490 nm.

Binding characteristics and cross-reactivity test by ELISA

For sensitivity and cross-reactivity study, the procedure was the same as above except that 100 µl antibody of the titer level concentration (Table

2) was pre-reacted with 100 μl known amounts of atrazine (0, 0.1, 1.0, 10 μg/ml). This mixture was incubated in pre-coated microtiter plate wells of six kinds of atrazine-KLH or simazine-KLH. For the cross-reactivity test, the triazines group chemicals: cyanazine, simetryne, ametryne, and cyanuric chloride, were used to evaluate the binding ability to the above antibodies. Trifluralin, which is not related with triazines in regard to their chemical structures, was also used to investigate the binding ability to antibodies.

RESULTS AND DISCUSSION

Synthesis of atrazine derivatives, immunogens and coating ligands

As shown in Figure 1, six kinds of carboxylic derivatives of triazines were synthesized by the method of Goodrow and Hammock (6).

We obtained three simazine derivative of 1a, 2a, 2b from simazine and cyanuric chloride and three atrazine derivatives of 1b, 2c, and 2d from atrazine and cyanuric chloride. After recrystallization, the ^{1}H-NMR spectra and melting point of each carboxylic derivative are determined to confirm the derivatization. The characteristics of ^{1}H-NMR spectra and melting points are as follows.

1a: 3-[2,4-bis(ethylamino)-1,3,5-triazine-6-yl thio] propanoic acid; mp = 179.8, ^{1}H-NMR (DMSO-d_6) δ 12.2 (br, 1 H, CO_2H), 7.1 (br, 2 H, 2 NH), 3.2 (m, 6 H, CH_2N), 2.60 (t, 2 H, CH_2CO_2), 1.06 (t, 6 H, 2 CH_3).

1b: 3-[2-(1-methylethyl)amino-4-(ethylamino)- 1,3,5-triazine-6-yl thio] thiopropanoic acid; mp = 163.6; ^{1}H-NMR (DMSO-d_6) δ 12.1 (br, 1 H, CO_2H), 7.0 (br, 2 H, 2 NH), 4.0 (m, 1 H, CH), 3.1 (m, 4 H, CH_2S, CH_2N), 2.57 (t, 2 H, CH_2CO_2), 1.1 (m, 9 H, 3 CH_3).

1c: [(4,6-dichloro-1,3,5-triazine-2yl) N-ethyl] amine; mp = 110.2; ^{1}H-NMR ($CDCl_3$) δ 6.7 (br, 1 H, NH), 3.53, 3.47 (two q, 2 H, CH_2), 1.24 (t, 2 H, CH_3).

1d: [(4,6-dichloro-1,3,5-triazine-2yl) N-(1-methylethyl)] amine; ^{1}H-NMR ($CDCl_3$) δ 5.9 (br, 1 H, NH), 4.2 (m, 1 H CH), 1.25 (d, 6 H, 2 CH_3).

2a: N-[2-(ethylamino)-6-chloro-1,3,5-triazine-4-yl] β -alanine; mp = 169.5; ^{1}H-NMR (DMSO-d_6) δ 7.7 (m, 2 H, 2 NH), 3.3 (m, 4 H, 2 AH_2N), 2.48 (t, 2 H, AH_2CO_2), 1.18, 1.15 (two t, 3 H, AH_3).

2b: 6-[2-(ethylamino)-6-chloro-1,3,5-triazine-4-yl] amino hexanoic acid; mp = 166.5, ^{1}H-NMR (DMSO-d_6) δ 11.8 (br, 1 H, CO_2H), 7.6 (br, 2 H, 2 NH), 3.2 (m, 4 H, 2 CH_2N), 2.18 (t, 2 H, CH_2CO_2), 1.4 (m, 6 H $CH_2CH_2CH_2$), 1.07 (two t, 3 H, CH_3).

2c: N-[2-(1-methylethyl) amino-6-chloro-1,3,5-triazine-4-yl] β -alanine; mp = 170.3, ^{1}H-NMR (DMSO-d_6) δ 7.8 (m, 2 H, 2 NH), 4.1 (m, 1 H, CH), 3.4 (m, 2 H, 2 NH), 2.5 (m 2 H, CH_2CO_2), 1.10, 1.13 (two d, 6 H, 2 CH_3).

2d: 6-[2-(1-methylethyl)amino-6-chloro-1,3,5-triazine-2-yl amino] hexanoic acid; mp = 164.5, ^{1}H-NMR (DMSO-d_6) δ 11.6 (br, 1 H, CO_2H), 7.4 (m,

2 H, 2 NH), 3.9 (m, 1 H, CH), 3.1 (m, 2 H, $CH_2CH_2CH_2$), 1.10 (two d, 6 H, 2 CH_3).

The immunogens were synthesized from 1b by conjugating BSA to the carboxylic moiety. Other ligand derivatives (1a, 1c, 1d, 2a, 2b, 2c, 2d) were conjugated with KLH for the selective use of coating microplate well for ELISA.

Preparation of monoclonal antibodies specific to atrazine derivatives

Five hybridoma cell lines (MAb 1H5, 2A11, 2F9, 2G5, and 4B9) were obtained from the fusion of spleen cells immunized with atrazine-BSA (1b-BSA conjugate). Figure 2 shows the competitive inhibition test of the supernatant of cloned hybridoma cell lines with various coated ligands (1a, 1b, 2a, 2b, 2c, and 2d) on the microtiter plate wells. Each cell line showed a different atrazine displacement reaction depending on the coated ligand on the microtiter plate. MAb 4B9 showed the most sensitive displacement reactions of atrazine with a 2b-KLH coated plate. But other cell lines of MAb 1H5 and MAb 2G5 showed similar diplacement reaction of atrazine with 2c-KLH and 1b-KLH coated plate, respectively. The results indicate that monoclonal antibodies obtained from each hybridoma cell line have distinctive epitope for the atrazine reaction.

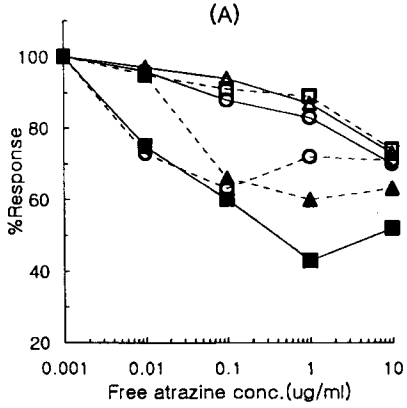
The amount of ascitic fluid formed by inoculating hybridoma cell lines was varied from 1.8ml (MAb 2G5) to 5.3ml (MAb 4B9) per mouse and the

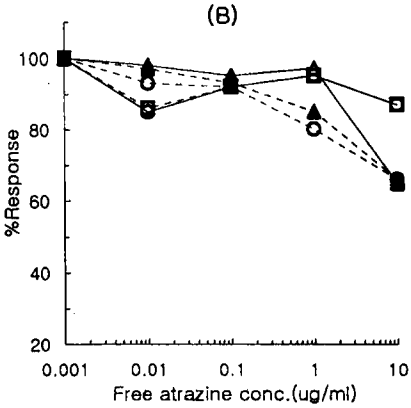
amount of immunoglobulin per milliliter of ascitic fluid was varied from 0.35 mg (MAb 4B9) to 0.65 mg (MAb 1H5). Even though MAb 4B9 produced the largest volume of ascitic fluid per mouse, its antibody content is lowest among five hybridoma cell lines. The purity of the protein A purified antibody was determined by 12% SDS-PAGE showing heavy chain (~50 kDa) and light chain (~23 kDa) for each clone of monoclonal antibody. The expected molecular weight of light chain was approximately 25 kDa and a low MW of light chain may reflect the glycosylation difference between antibodies. The purification results are summarized in Table 1.

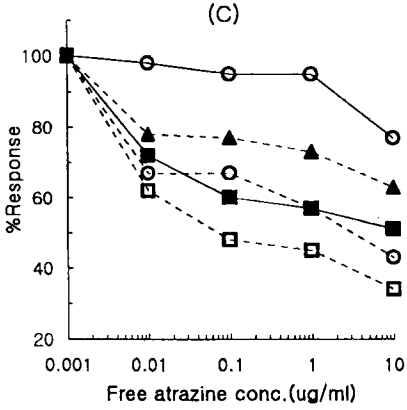
Titration of antibodies by competitive ELISA

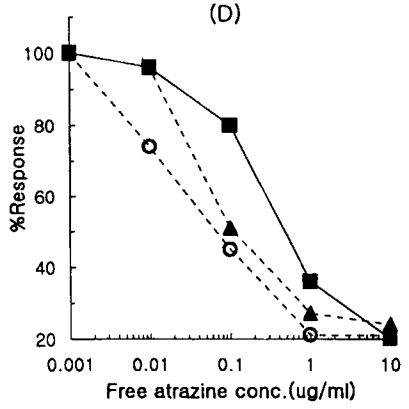
Table 2 are summary of the titer level of protein A-purified antibodies using coated ligand on the microtiter plate by the competitive ELISA. The coated ligands are variously derivatized atrazine-KLH (1a, 2a, and 2b) and simazine-KLH (1b, 2c, and 2d). The titer level expressed as the antibody concentration showing absorbance 2.0 at 490 nm using ligand-KLH coated on the microtiter plate.

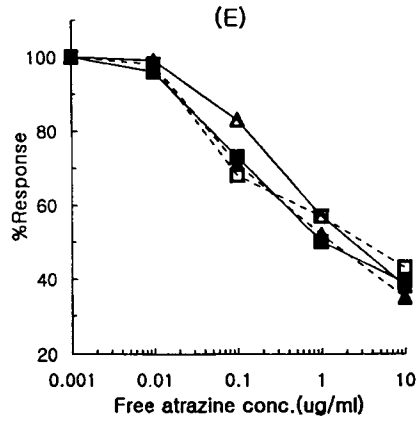
The purified MAb 2G5, unexpectedly, could not produce titration curves for all six kinds of coated ligands (1a, 2a, 2b, 1b, 2c, 2d), which was coupled with KLH, even though it produced atrazine reactivity during the screening steps of the antibody-positive hybridoma cell lines (Figure 2(D)). We expected that the best titer level of each monoclonal antibody is to be with 1b-KLH











coated plate because monoclonal antibodies were produced with 1b-BSA which is atrazine derivative. However, monoclonal antibodies (MAb 1H5, 2A11, 2F9, and 4B9), unexpectedly, showed much better titer level with 1a-KLH, a simazine derivative. The highest titer level was observed in clone MAb 2F9 and MAb 4B9 with 1a-KLH coated microplate and titer level of MAb 2F9 and MAb 4B9 was 0.02 μg and 0.03μg, respectively.

The binding characteristics and specificity of antibodies

The binding characteristics of purified antibodies were determined for free atrazine using various triazine ligand-coated plates by competitive ELISA.

FIGURE 2. Antibody screening in supernatants of hybridoma cell lines. Six kinds of derivatized ligand-KLH conjugates (1 μg/ml, 200 μl/well) were coated on the microplate and 3% BSA was post-coatd. free atrazine (100 μl) and supernatant of each hybridoma cell line (100 μl) was added and incubated for 2 hours at RT. After washing, anti-mouse IgG-HRP conjugate (1:2,000 diluted, 200 μl) was incubated for 2 hours at RT. After wash with 10 mM PBST 3 times, OPD substrate (200 μl) was incubated for 10 minutes at RT and 50 μl 4N H_2SO_4 quenching solution added. The absorbance was read at 490 nm. The antibody binding activity was determined by the response difference of competitive ELISA between the coated ligand and free atrazine for the antibody binding sites in the supernatant of each hybridoma cell line. (A): MAb 1H5, (B): MAb 2A11, (C): MAb 2F9, (D): MAb 2G5, (E): MAb 4B9. Coating ligand: 1a-KLH (—O—), 1b-KLH (--O--), 2a-KLH (—△—), 2b-KLH (--□--), 2c-KLH (—■—), 2d-KLH (--▲--).

TABLE 1

Production of ascitic fluids and purification of monoclonal antibodies

MAb	# of mouse Inoculated	Total asc fld (ml)	Asc fld /mouse (ml)	Purified IgG (mg)	Yield (IgG/asc fld) (mg/ml)
MAb 1H5	4	16.0	4.0	10.4	0.65
MAb 2A11	3	7.0	2.3	3.6	0.51
MAb 2F9	4	19.0	4.7	10.5	0.55
MAb 2G5	3	5.5	1.8	2.3	0.42
MAb 4B9	4	21.3	5.3	7.4	0.35

Immunogen of antibodies were 1b-BSA conjugate

TABLE 2

Titer levels of monoclonal antibodies for the derivatized ligands, atrazine-KLH or simazine-KLH, coated on the microtiter plate by competitive ELISA

	Titer level of MAb (μg/ml)					
Coating ligand / MAb	1a-KLH (C3)	1b-KLH (C3)	2a-KLH (C3)	2b-KLH (C6)	2c-KLH (C3)	2d-KLH (C6)
MAb 1H5	0.2	1.3	0.2	0.7	0.4	0.6
MAb 2A11	0.15	0.7	0.1	3.0	0.2	0.7
MAb 2F9	0.02	0.3	1.0	0.5	3.0	0.2
MAb 2G5	n/d	n/d	n/d	n/d	n/d	n/d
MAb 4B9	0.03	0.2	1.0	3.0	2.0	0.15

n/d: cannot be determined.
C3: spacer length (3 carbon derivative of ligand for KLH conjugation).
C6: spacer length (6 carbon derivative of ligand for KLH conjugation).

TABLE 3

The effect of coating ligands on the sensitivity variation of atrazine detection by the competitive ELISA

	Sensitivity for atrazine (μg/ml)					
Coating ligand / MAb	1a-KLH (C3)	1b-KLH (C3)	2a-KLH (C3)	2b-KLH (C6)	2c-KLH (C3)	2d-KLH (C6)
MAb 1H5	n/d	4.0	n/d	n/d	n/d	6
MAb 2A11	n/d	n/d	n/d	n/d	n/d	n/d
MAb 2F9	n/d	5.0	0.15	0.04	1.3	40
MAb 4B9	n/d	3.0	0.2	0.06	0.08	25

n/d: cannot be determined
C3 and C6: spacer length

Antibodies (MAb 1H5, 2A11, 2F9, and 4B9) were pre-reacted with free atrazine, and applied to each ligand-KLH coated plate (ligands: 2a, 2c, 2d, 1a, 2b, and 2c). The assay sensitivities were determined by means of atrazine concentration in which the absorbance was reduced by 20% due to the competitive binding of atrazine to the antibody. The results of binding sensitivities were summarized in Table 3. The MAb 2A11, which showed weak antibody reactivity in the screening steps of hybridoma cell lines, did not show any reacivity with free atrazine. We observed that the binding reactivity of purified antibody were, in general, weaker than supernatant produced from the same hybridoma cell line. We found that the titer level of hybridoma supernatants, 1H5, 2A11, 2F5, 2G5, and 4B9 showed at dilution folds of 3, 9, 729, 1, and 9 respectively. These values correspond to 0.2, 0.05, 0.0008, 0.4,

0.03 μg/ml of purified monoclonal antibodies according to calculations based on the antibody purification yields in Table 1. Comparing these values to the titer levels of purified monoclonal antibodies in Table 2, the monoclonal antibodies 1H5 and 4B9 showed similar titer levels, but 2A11 showed 3 times less binding activity and 2F9 showed 25 times less binding activity. In the case of monoclonal antibody 2G5, the titer level could not be determined using purified monoclonal antibody with microtiter plates coated with 2a-KLH, 2b-KLH and 2c-KLH, but the supernatant showed titer levels at the dilution folds of 81, 81 and 27 respectively. These results indicate that some of the antibodies can be affected in their binding characteristics after the protein A purification step, and their binding characteristics also depend on the ligand used to coat the microtiter plates. These phenomena may be due to the subtype differences of mouse immunoglobulin which showed binding affinity differences during the protein A purification step.

The best titer level was observed in clone MAb 2F9 and MAb 4B9 with 1a-KLH coated microplate and titer level of MAb 2F9 and MAb 4B9 was 0.02 μg and 0.03μg, respectively. The high titered antibodies, MAb 2F9 and MAb 4B9, showed better sensitivity response than other monoclonal antibody clones, but the coating ligand were not identical for the best response between sensitivity and titer level. The high titer levels of monoclonal antibodies were showed with 1a-KLH coated plate, but high sensitivity with 2b-KLH coated plate, and no sensitivity response was seen with 1a-KLH coated plate. This

result indicates that higher titer does not necessarily lead to high assay sensitivity.

Comparison of sensitivity response was made in terms of length of spacer arms. MAb 2F9 and MAb 4B9 were 3 times better with 2b-KLH coated microplate than 2a-KLH coated plate, where 2a-KLH was derivatized with 6 carbon space arms and 2a-KLH derivatized with 3 carbons. Similar results were reported in ELISA using atrazine-HRP tracer indicating that the longer space arms resulted in a more sensitive assay when conjugated to HRP (9).

The cross-reactivities were determined for the MAb 1H5, 2F9, and 4B9 which showed reactivity with atrazine. The coating ligand was selected based on the results of binding chracteristics, in which the best sensitivity of atrazine reaction was determined. The 1b-KLH coated plate was used for the cross-reactivity study of MAb 1H5 and 2b-KLH coated plate was used for MAb 2F9 and MAb 4B9. The results are summarized in Figure 3. MAb 1H5 showed very low response to atrazine and no response to simazine. However, MAb 2F9 and MAb 4B9 showed selective responses to atrazine group compounds, such as atrazine (0.04 and 0.06 μg/ml, respectively, at 80% response), ametryn (0.03 and 0.15 μg/ml, respectively), and cyanazine (0.4 and 0.05 μg/ml, respectively), without cross-reacting to simazine and cyanuric chloride. Each structure of tested triazine group compound was compared and cross-reactivity was summarized in Table 4.

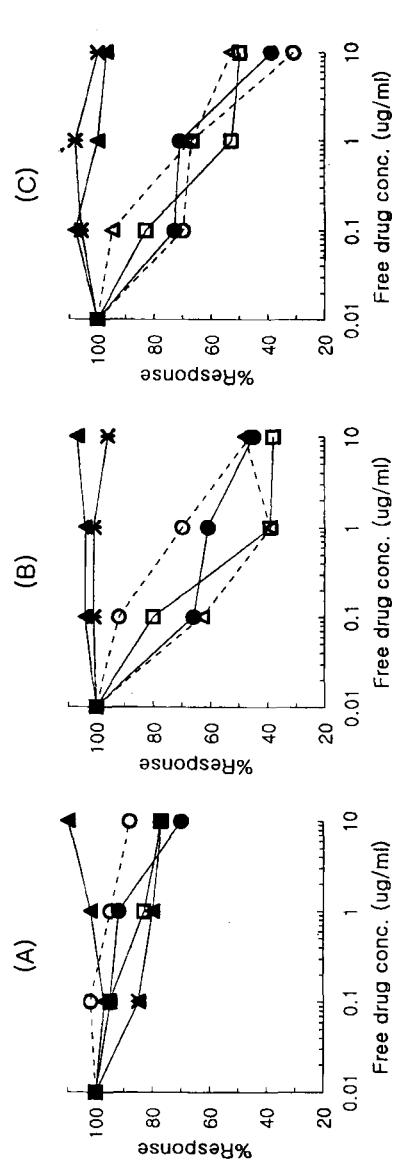
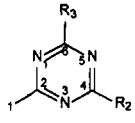
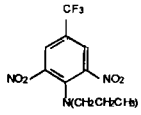


FIGURE 3. Cross-reactivity study for monoclonal antibodies by competitive ELISA. Microtiter plate was coated withligand (200 µl, 1 µg/ml) of best sensitivity (1b-KLH for MAb 1H5 clone experiment, 2b-KLH for MAb 2F9 clone and MAb 4B9 clone). After post-coating with 3% BSA, 100 µl of triazines for cross-reactivity study and 100 µl each monoclonal antibody of titer level (MAb 1H5: 1.3 µg/ml, MAb 2F9: 0.5 µg/ml and MAb 4B9: 3.0 µg/ml) were added and incubated for 2 hours at RT. The rest of the procedure is the same as described in Figure 2. (A): MAb 1H5; (B): MAb 2F9; (C): MAb 4B9. Cross-reacting substance: atrazine (—●—), ametryne (—□—), simetryne (--Δ--), simazine (—▲—), cyanazine (--O--), cyanuric chloride (—*—).

TABLE 4

Cross-reactivity with monoclonal antibodies with various triazine compounds

	1,3,5-triazine ring			Cross-reactivity(%)	
	R1	R2	R3	MAb 2F9	MAb 4B9
Atrazine	$-NHCH(CH_3)_2$	$-NHCH_2CH_3$	$-Cl$	100	100
Ametryne	$-NHCH(CH_3)_2$	$-NHCH_2CH_3$	$-SCH_3$	40	40
Cyanzine	$-NHC(CH_3)_2$ CN	$-NHCH_2CH_3$	$-Cl$	0	120
Simazine	$-NHCH_2CH_3$	$-NHCH_2CH_3$	$-Cl$	0	0
Simetryne	$-NHCH_2CH_3$	$-NHCH_2CH_3$	$-SCH_3$	133	7
Cyanuric Chloride	$-Cl$	$-Cl$	$-Cl$	0	0
Trifluralin				0	0

In conclusion, two monoclonal antibodies of MAb 2F9 and 4B9 specific to the atrazine group were selected using an immunogen of atrazine conjugate (2b-BSA). The antibody responses of MAb 2F9 and 4B9, in terms of sensitivity and cross-reactivity, were the best choices for atrazine and its related compounds in immunoassay using simazine derivative-KLH (2b-KLH) coated plate. Therefore, these antibodies can be utilized for various studies of screening test of atrazine group compounds for monitoring in ground water and monitoring of contaminants of produce.

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Abbreviations used; MAb, monoclonal antibody; BSA, bovine serum albumin; KLH, keyhole limpet haemocyanin; ELISA, enzyme-linked immunosorbent assay; OPD, *o*-phenylenediamine; PBST, phosphate buffered saline containing 0.05% tween-20; RT, room temperature.

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