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Evaluation of a water-soluble adjuvant for the development of monoclonal antibodies against small-molecule compounds*

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Abstract: A water-soluble adjuvant named QuickAntibody (QA) was introduced into the procedure of mouse immunization for the development of hapten-specific monoclonal antibodies (mAbs), using four kinds of pesticides as model compounds. Compared with conventional Freund's adjuvants, QA treatments offered relatively low but acceptable antiserum titers after three inoculations, gave little adverse effects to the experimental animals, and were preferable in harvesting splenocytes during the steps of cell fusion. Afterwards, hybridomas from the QA group were prepared and screened by both non-competitive and competitive indirect enzyme-linked immunosorbent assays (ELISAs). The efficiency of gaining immune-positive hybridomas was satisfactory, and the resultant mAbs showed sensitivities (half maximal inhibitory concentration (IC_{50})) of 0.91, 2.46, 3.72, and 6.22 ng/ml to triazophos, parathion, chlorpyrifos, and fenpropathrin, respectively. Additionally, the performance of QA adjuvant was further confirmed by acquiring a high-affinity mAb against okadaic acid (IC_{50} of 0.36 ng/ml) after three immunizations. These newly developed mAbs showed similar or even better sensitivities compared with previously reported mAbs specific to the corresponding analytes. This study suggested that the easy-to-use adjuvant could be applicable to the efficient generation of highly sensitive mAbs against small compounds.

Key words: Water-soluble adjuvant, Monoclonal antibody, Hapten-specific antibody, High affinity
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1 Introduction

Naturally occurring antibodies, especially monoclonal antibodies (mAbs), have proved to be versatile biological tools in modern bioanalytical methods for the determination of both macromolecules and low-molecular-weight compounds (e.g. drugs of abuse, pesticides, mycotoxins, antibiotics, and other contaminants in environmental and food samples). By comparison, the development of high-affinity mAbs against small molecules (haptens) is relatively difficult and usually needs long preparation time (more than four immunizations), because haptens have no immunogenicity and must firstly be coupled to carrier proteins to sufficiently provoke an immune response. Additionally, compared with the chance to obtain high-affinity antibodies against large molecules (e.g. protein antigens), the chance to get the effective mAbs with free hapten-binding ability is relatively low. For instance, only three hybridomas of interest were found among a total of 8700 cultured wells from ten fusions in a previous report on the production of mAbs against oxytetracycline, an antibiotic with the molecular weight of 460.4 Da (Wongtangprasert *et al*., 2014).

Immunologic adjuvants are essential for immunization. In cases where the antigen is of weak immunogenicity or has a very limited availability, the type and role of adjuvant become key components for

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producing excellent antibodies (Stils, 2005). For research purposes, Freund's adjuvant (complete or incomplete) is still the most extensively used and effective for making the water-in-oil emulsion for immunization of laboratory animals. Unfortunately, the water-in-oil emulsion tends to denature protein antigens, thus hindering the induction of antibodies against specific epitopes (Algate *et al*., 2013). Moreover, it is inherently toxic and may cause abscesses and granulomas at injection sites, resulting in high concern for research animals. Hence, many researchers and animal care facilities encourage the use of other adjuvants. Ideally, an adjuvant should support an immune response against a wide variety of antigen structures even if exhibiting little antigenicity. Up to now, some alternatives (e.g. TiterMax™, Ribi™, and Gerbu™ adjuvants) have been invented to generate a potent immune response with significantly reduced toxicity (Stils, 2005; Algate *et al*., 2013), but most of the evaluation studies were focused on developing antibodies to macromolecules. Only a few investigations have been carried out to find replacements for Freund's adjuvants in the production of hapten-specific antibodies (Erhard *et al*., 1991; Fodey *et al*., 2008; George *et al*., 2012; Maquieira *et al*., 2012; Parra *et al*., 2013).

Recently, a novel proprietary adjuvant named QuickAntibody (QA), invented by Beijing Biodragon Immunotechnologies Co. in China, was designed specifically for eliciting monoclonal and polyclonal antibodies in mice. It is formulated with pathogen-associated molecular patterns containing several Toll-like receptor ligands and cationic polymers to activate cytosolic pathogen-recognition receptors, acting as an immune modulator. According to the manufacturer, QA is a ready-to-use water-compatible solution that can be easily mixed with any antigen before immunization through the simple operation of intra-muscular injection. Until now, it has mainly been used for the preparation of mouse antibodies against different types of bio-antigens (Ruan *et al*., 2013; Zhang *et al*., 2013c; Zhao M. *et al*., 2013; He *et al*., 2014; Zhao Z. *et al*., 2014; Wang *et al*., 2015), while the application of QA adjuvant in the preparation of mAbs against small-molecule compounds is limited to a few reports from one research group (Chen *et al*., 2013; Zhang *et al*., 2013a; 2013b). Because the new water-soluble adjuvant was initially invented for macromolecular bio-antigens, to establish whether it is suitable for rapid development of mAbs towards various haptens requires many trials.

The aim of this work was to evaluate QA as a potential candidate for the efficient generation of highly sensitive mAbs against small molecules. Several kinds of agricultural chemicals were taken as the target analytes. Additionally, mAbs for okadaic acid (OA), a marine biotoxin, were also developed by using the QA adjuvant, to explore its applicability.

2 Materials and methods

2.1 Reagents and materials

Triazophos, parathion, chlorpyrifos, fenpropathrin, and other related pesticide standards were obtained from Agro-environmental Protection Institute, Ministry of Agriculture (Tianjin, China). OA standard was purchased from Algal Science Inc. (Taoyuan, Taiwan, China). Complete and incomplete Freund's adjuvants, ovalbumin (OVA), bovine serum albumin (BSA), pristane, and rabbit anti-mouse IgG secondary antibody labeled with horseradish peroxidase (SecAb-HRP) were from Sigma-Aldrich (St. Louis, MO, USA). QA adjuvant was supplied by Kang Biquan Biotechnology (Beijing, China). *O*-phenylenediamine (OPD) and Tween 20 were purchased from Shanghai Chemical Reagents Company (China). Blue carrier protein (BCP) was purchased from Thermo Scientific (Rockford, USA). Ultra-pure water was acquired from a Milli-Q purification system (Millipore, Bedford, USA). Phosphate-buffered saline (PBS; 10 mmol/L, pH 7.4), carbonate-buffered saline (CBS; 50 mmol/L, pH 9.5), and OPD substrate solution (10 mg of OPD and 10 μl of 30% H_2O_2 diluted with 25 ml of phosphate-citrate buffer at pH 5.4) were self-prepared. All other reagents were of analytical grade unless specified otherwise.

MaxiSorp™ F96-well polystyrene microplates and different kinds of cell culture plates were obtained from Nalge Nunc International (Roskilde, Denmark). Protein concentrators (10 kDa molecular weight cut-off (MWCO), 20 ml) were purchased from Pall (New York, USA). Isotype classifications of the mAbs were performed by a commercially available kit from Thermo Scientific (Rockford, USA). The mouse SP2/0 myeloma cells were purchased from the Cell Bank at the Chinese Academy of Sciences (Shanghai, China).

The functionalized derivatives (haptens) of target pesticides were designed and synthesized as described in our published research (Gui *et al*., 2006; Jin *et al*., 2006; Liu *et al*., 2007; Liang *et al*., 2013). Fig. 1 lists the chemical structures of related compounds used in the present work.

2.2 Animals

Female Balb/c mice and F1 hybrid mice were provided by Shanghai Lab Animal Research Center (China). They were housed under standard conditions of temperature (20–25 °C) and humidity (40%–60%) with a 12-h light/dark cycle, and had free access to drinking water and a commercial pellet diet. Animal manipulations were carried out in compliance with Zhejiang University guidelines for the welfare of experimental animals.

2.3 Preparation of hapten-protein conjugates

For pesticide immunogens, haptens were covalently attached to the lysine groups of BSA by the active ester method. To synthesize the hapten-OVA conjugates used as coating detective antigens, the mixed anhydride method was adopted using isobutyl chloroformate as the coupling reagent. Conjugates of OA-BCP and OA-BSA respectively serving as the immunogen and the coating antigen were prepared according to a previous research method (Lu *et al*., 2012). Purification was performed by dialyzing extensively with PBS, followed by treatment with a protein concentrator.

2.4 Immunization strategy

All hapten-protein immunogens in this study were administered at a dose of 50 μg per mouse for each inoculation, with the total injection volume of 100 μl. The injection solution was freshly prepared by simply mixing the immunogen in sterile saline with an equal volume of QA adjuvant, according to the QA product manual (the adjuvant should be shaken well before use, because some precipitation may appear after storage). For the initial immunization, three 6-week-old female Balb/c mice were given a single intramuscular injection. Three weeks later, the mice were boosted, and the third inoculation was given after another 14 d. One week later, mice were tail-bled and titers of antisera were tested by indirect enzyme-linked immunosorbent assays (ELISAs) to evaluate the immune responses. The most representative

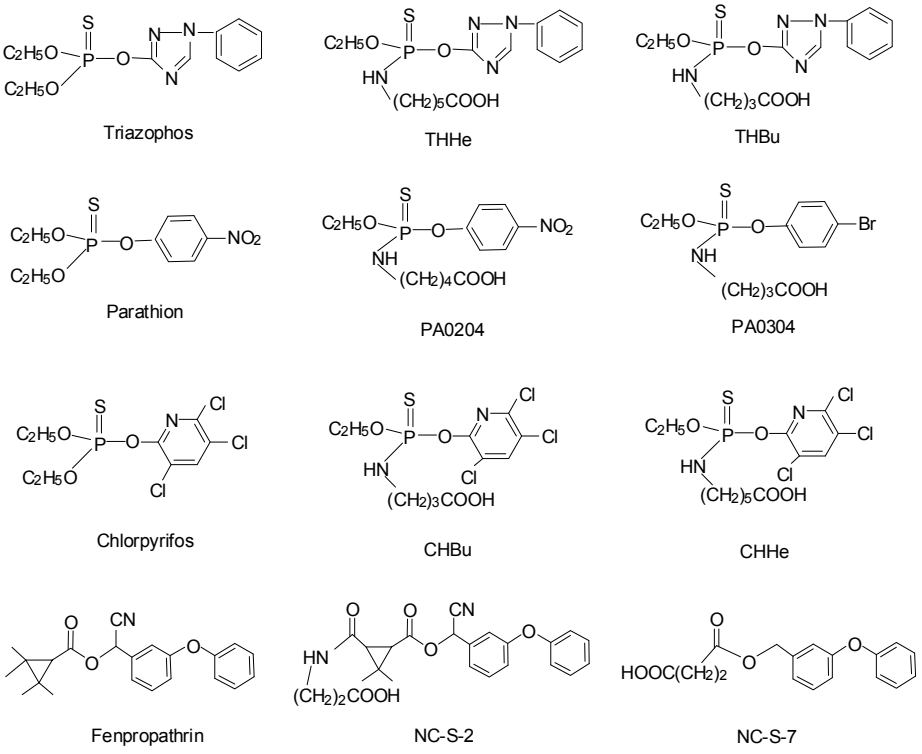

Fig. 1 Chemical structures of related compounds used in this work

mouse that produced antibodies with both satisfying titer and the best recognition toward the test substance was selected for cell fusion.

As control, the immune responses of mice immunized with conventional Freund's adjuvants were also evaluated. Mice were administered with the similar immunization strategy and the same dose of immunogens as mentioned above. Primary immunization was carried out by multi-site subcutaneous injection using complete Freund's adjuvant for emulsification, while subsequent boosters were administered by intraperitoneal injection using incomplete Freund's adjuvant.

2.5 Cell fusion and hybridoma screening

After a resting period of two weeks from the last injection with adjuvant, the mouse chosen to be the spleen donor received a final intraperitoneal booster with 50 μg of the immunogen dissolved in sterile saline (without adjuvant). Three days later, the spleen cells were collected and fused with SP2/0 myeloma cells. Fusion procedures were carried out essentially based on standard procedures and some details previously described (Wang *et al*., 2009).

Around 12–14 d after cell fusion, non-competitive indirect ELISAs were used for primary screening of hybridomas. Wells showing the absorbance values higher than 0.5 were selected and filled with fresh medium. The next day, they were rechecked by competitive indirect ELISAs to test the ability to recognize the target compounds. Wells of hybridomas giving strong inhibition (more than 50%) by the analytes at 1 μg/ml and maintaining absorption values were expanded into 24-well culture plates. The supernatants were re-screened by ELISAs. Only those clones that still maintained high absorbance values, had strong affinity to the analytes, and displayed no cross-reactivity to OVA, were chosen for next-step selection (Zhang *et al*., 2009). Normally, the top three hybridomas for each analyte were subjected to subcloning for 4–6 cycles to get the final stable single-cell lines.

For large-scale production of mAbs, the representative clone for each pesticide was intraperitoneally inoculated into F1 hybrid mice (106 cells per mouse), which were pre-treated with pristane (0.5 ml per mouse). After 7–10 d, the ascites fluid was collected and then purified by caprylic acid-ammonium sulfate precipitation.

2.6 Indirect ELISA

All incubations were conducted at 37 °C, except for the coating step. After each incubation, plates were washed three times with PBST (0.05% Tween 20 in PBS, 300 μl/well) using a 96-channel washer (High-creation, Shenzhen, China). High-binding polystyrene microplates were filled with coating antigens diluted in CBS (100 μl/well) for incubation at 4 °C overnight. The next day, the plates were blocked with 2% (0.02 g/ml) skim milk in PBS (300 μl/well) for 30 min. For the competitive indirect ELISA, standard solutions of analytes were freshly prepared by PBS containing a certain percentage (v/v) of methanol as co-solvent, that is, 5% for triazophos, 10% for parathion, 20% for chlorpyrifos, and 30% for fenpropathrin, based on our previous work experience. Then, both blank control and analyte solutions were respectively added (50 μl/well), followed by adding 50 μl/well of antibodies diluted in PBS. After shaking and incubation for 1 h, 100 μl/well of SecAb-HRP (1:40000 (v/v) dilution in PBS) was added and incubated for 45 min. Then, 100 μl/well of freshly prepared OPD substrate solution was added. The reaction was stopped after 15 min with 2 mol/L sulphuric acid (50 μl/well). Finally, the absorbance (optical density at 490 nm, OD_{490}) was measured by a SpectraMax i3 microplate reader (Molecular Devices, Sunnyvale, USA).

2.7 Assay sensitivity test

Checkerboard titration was performed by competitive indirect ELISAs with a range of antibody and coating antigen dilutions. The combination of antibody and antigen dilutions which provided the greatest inhibition by adding the free analyte was ascertained to be optimal for subsequent use in the assay. Competitive standard curves were obtained by plotting the inhibition rate against the logarithm of analyte concentration. Simulation of standard curves was performed by means of Origin 8.5 software. The IC_{50} value (i.e., analyte concentration that caused a 50% reduction of binding between the antibody and the coating antigen) was determined to estimate the assay sensitivity.

2.8 Cross-reactivity study

To evaluate antibody specificity or assay selectivity, analogs of target pesticides were utilized to implement cross-reactivity studies. The IC_{50} of each

chemical was determined from its standard curve. Cross-reactivity (CR) values were calculated based on the following equation: CR=IC_{50} (target analyte)/ IC_{50} (analogue)×100%.

3 Results and discussion

3.1 Immunological response

The current study was undertaken to assess the efficacy of QA adjuvant for the rapid development of hapten-specific mAbs with high affinity, which could be applied to various detection methods for diverse chemical contaminants in the fields of food safety control and environmental monitoring. The primary indicator for evaluation was if QA could help to elicit a similar or enhanced immune response in the host animal without the adverse effects derived from Freund's adjuvants.

In the present work, the functionalized haptens for target pesticides were rationally designed according to our previous research results. Chemicals named THHe, PA0204, CHBu, and NC-S-2 were used as the immunizing haptens for triazophos (Gui *et al*., 2006), parathion (Wang *et al*., 2009), chlorpyrifos (Jin *et al*., 2006), and α-cyano pyrethroids (Liang *et al*., 2013), respectively. Moreover, a certain degree of hapten heterology is often employed to improve the detectability of competitive immunoassays for small-molecule analytes (Kim *et al*., 2003; Wang *et al*., 2011; Esteve-Turrillas *et al*., 2014). Herein, based on our experience, moderately heterologous haptens (THBu, PA0304, CHHe, and NC-S-7) were used to choose the optimal competitor/antibody combinations for the establishment of highly sensitive immunoassays and maybe also for hybridoma screening.

For primary testing of antiserum titers, homologous antigens were coated at 10 μg/ml for non-competitive indirect ELISAs and the antiserum dilutions offering OD_{490} close to 1.0 were set as the titers. Fig. 2 presents their mean values from three mice in each group after the third immunization. It was clear that, for each pesticide treatment, the QA group generally showed relatively lower titer than the Freund's group, but it was still acceptable. Apart from the titer measurement, the inhibition rate by the free analyte was further determined to see if useful antibody was produced, by adding a high concentration of the related pesticide in competitive indirect ELISAs. Because the antisera collected from mouse tails were very limited and our final purpose was to develop mAbs for these pesticides, working conditions of coating antigens and mouse antisera for ELISAs were not optimized, but just based on the fixed concentration of antigens at 10 μg/ml and the corresponding antiserum titers. According to the inhibition results caused by related pesticides (Table 1), there were no significant differences between the two adjuvant

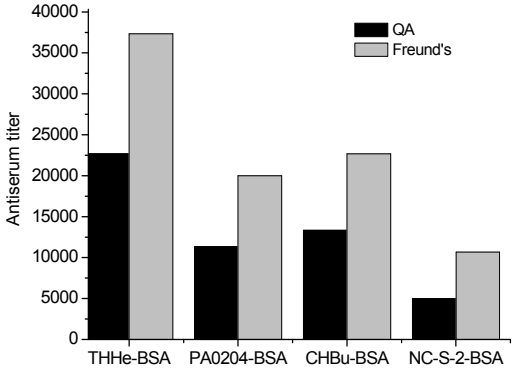

Fig. 2 Antiserum titer of each immunization group after the third inoculation

Table 1 Primary characterization of antiserum affinities to free analytes using homologous and heterologous antigens coated for competitive indirect ELISAs[a]

Immunogen	Adjuvant	Coating antigen	Inhibition rate (%)
THHe-BSA	QA	THHe-OVA	58.7±8.2
		THBu-OVA	79.4±9.1
	Freund's	THHe-OVA	61.2±6.7
		THBu-OVA	85.5±8.7
PA0204-BSA	QA	PA0204-OVA	54.3±6.5
		PA0304-OVA	67.7±9.3
	Freund's	PA0204-OVA	49.0±7.1
		PA0304-OVA	71.1±7.6
CHBu-BSA	QA	CHBu-OVA	36.2±4.8
		CHHe-OVA	43.0±4.0
	Freund's	CHBu-OVA	38.6±5.5
		CHHe-OVA	43.3±7.0
NC-S-2-BSA	QA	NC-S-2-OVA	15.7±5.4
		NC-S-7-OVA	−
	Freund's	NC-S-2-OVA	18.6±5.9
		NC-S-7-OVA	−

[a] In competitive indirect ELISAs, 1 μg/ml of triazophos or parathion and 2 μg/ml of chlorpyrifos or fenpropathrin were individually used for inhibition measurement. The inhibition rate is the average value± standard deviation (SD) from three mice in each group

groups in all cases (Student's *t*-test, $P>0.05$), which seemed promising for gaining effective mAbs under the administration of QA adjuvant. Overall, the performance of QA was comparable to that of traditional Freund's adjuvants based on the polyclonal antibody characteristics, although not as good as the manufacturer had announced (higher antibody titer and affinity).

Additionally, heterologous coating antigens were also implemented for comparison. As for triazophos and parathion, using THBu-OVA and PA0304-OVA as the competitors indeed promoted the assay sensitivities for the target pesticides in the cases of both adjuvants, whereas the heterologous ELISA gave no substantial improvement in the case of chlorpyrifos. For the NC-S-2-BSA group, it was found that the resultant antiserum could only well recognize the homologous antigen, and the most notable inhibition was induced by fenpropathrin among several α-cyano pyrethroids such as deltamethrin, cypermethrin, cyhalothrin, and fenvalerate. This result contradicted our initial expectation, since the preceding rabbit polyclonal antibodies generated from NC-S-2-BSA could recognize the heterologous coating antigen NC-S-7-OVA, and the established ELISA displayed a close affinity to a few α-cyano pyrethroids (Liang *et al*., 2013). It must be associated with the fact that antibodies produced from different animals can have different recognition spectra (specificity). Based on these antiserum results, except for NC-S-2-BSA hybridomas, indirect ELISAs coated with heterologous antigens could be used for hybridoma screening, in order to reduce the proportion of false positives caused by the spacer arms or the whole hapten-arm regions (Guo *et al*., 2014).

3.2 Animal health

When samples of tail blood were collected, mouse reactions to the immunogens were evaluated by monitoring the injection sites for the presence of adverse effects such as inflammation, abscess, or granuloma. Through the whole period of trials, none of the mice administered with QA was found to have severe adverse reactions in all pesticide groups, except that two mice appeared to have a little swelling at the intra-muscular injection sites. In contrast, granulomas were observed at almost all subcutaneous sites of each mouse injected with Freund's adjuvant. Fortunately, each mouse remained active until the study was completed. Therefore, QA seemed to be a relatively safe adjuvant with negligible side-effects in mice. It may have the potential to be applied in making vaccines for drugs of abuse and addiction. The theoretical basis of such vaccines is to induce antibodies that capture the drugs in the blood and thus are not able to cross the blood-brain barrier, thereby preventing psychoactive effects (Alving *et al*., 2014). Since injection of T cell-independent haptenic drugs hardly triggers memory recall responses, a potent and safe adjuvant for vaccine immunization plays a critical role (Alving *et al*., 2014).

3.3 Cell fusion and hybridoma selection

The objective of this study was to assess the performance of QA on the production of highly sensitive mouse mAbs toward small molecules, in that the adjuvant manufacturer did not conduct these trials before commercialization. Thus, apart from antiserum titer and affinity, the efficiency of generating hapten-specific hybridoma clones also should be examined and taken into account.

Since cell fusion per se is a technically crucial part of this study, all the fusion procedures were manipulated by one person to minimize individual differences. After antiserum screening, the most representative mouse from each group was selected and sacrificed for cell fusion. It is normal that, in cases of Freund's mice, it takes a few minutes to get rid of some white adipose tissues surrounding the spleens before the harvest of splenocytes. In fact, the spleens looked very clean and were more easily isolated from QA mice. This fact might be related to the intramuscular injections for QA treatment, which were different from the intraperitoneal injections for Freund's mice. This could be another advantage of QA over Freund's adjuvants in the aspect of easy handling for splenocyte harvesting.

Due to a large number of spleen cells, at least ten 96-well plates were used to distribute the fused cell suspension. The fusion rates were satisfactory, since more than 80% of wells contained at least one cluster of hybridomas. After checking by both non-competitive and competitive ELISAs (antigens coated at 10 μg/ml), only wells of hybridomas with satisfying affinity to analytes were selected for expansion, so as to decrease unnecessary workload and exorbitant use of

precious materials in the following steps. We realized that using a high concentration at 10 μg/ml for the coating antigen would not favor the competition, and hence some clones with a certain affinity to the free pesticides would be neglected. However, from another point of view, only clones with high affinity to the target analytes could be quickly selected as the useful hybridomas, reducing the number of clones with fairly low affinity.

Table 2 shows the hybridoma screening results from mice administered with QA adjuvant. For each pesticide, more than 20 wells were found to be strongly positive ($OD_{490}\geq0.5$). Among them, at least 30% of wells displayed great inhibition (more than 50% by 1 μg/ml of analyte). This was unexpected, but interesting, although the antiserum titer of QA group was not as high as that of Freund's group. The finding was consistent with a systematically comparative study of seven adjuvants, reporting that there was no correlation between the polyclonal antibody titer and the frequency of immune-positive clones (Ferber *et al*., 1999). Furthermore, on many occasions of producing antibodies against haptens, it does not follow that higher titer indicates greater sensitivity to the free analyte (Fodey *et al*., 2008; George *et al*., 2012). Overall, the QA adjuvant could be used to generate hapten-specific hybridoma clones with a satisfactorily efficient rate.

3.4 Antibody characterization

After subcloning and re-checking, the most sensitive and steady hybridoma clone representative for each pesticide was injected into F1 hybrid mice for ascites production. Isotypes of the purified mAbs were mostly IgG1 heavy chain and *kappa* light chain (Table 3).

Under suitable working concentrations of immunoreagents, the sensitivities of mAbs were measured by indirect competitive ELISAs with both homologous and heterologous coating antigens. As shown in Fig. 3, antibody sensitivities were significantly enhanced in heterologous formats for all three organophosphorus pesticides. For chlorpyrifos, improvement of one order of magnitude was observed, with IC_{50} value decreasing from 25.15 ng/ml (CHBu-OVA-based ELISA) to 3.72 ng/ml (CHHe-OVA-based ELISA). Table 3 summarizes the mAbs' sensitivities (IC_{50}) to the four pesticides in this study and in previous work, based on competitive indirect ELISAs. It was pleasing to find that the newly developed mAbs induced by the QA system could provide similar or even better sensitivities to the target analytes, compared with results from other publications (Manclús *et al*., 1996; Jin *et al*., 2008; Wang *et al*., 2009; Kim *et al*., 2011; Chen *et al*., 2014; Li *et al*., 2015), in which Freund's adjuvants and the traditional immunization strategy were used (mostly with the dose of 100 μg per mouse for each inoculation and 4–5 immunizations at 2- or 3-week intervals). These results were probably ascribed to features of the QA adjuvant, such as being water-compatible and with little influence of natural antigen conformation. Therefore, the new water-soluble adjuvant proved to be suitable for the efficient production of high-affinity mAbs against small chemicals.

Table 2 Hybridoma screening results from representative QA mice

Pesticide	Immunogen	Wells of $OD_{490}\geq0.5$	Wells of inhibition ≥50%[a]	Efficient rate (%)
Triazophos	THHe-BSA	24	19	79.2
Parathion	PA0204-BSA	95	30	31.6
Chlorpyrifos	CHBu-BSA	85	28	32.9
Fenpropathrin	NC-S-2-BSA	27	8	29.6

[a] Inhibition by 1 μg/ml of target pesticide

Table 3 Isotypes of newly developed mAbs and sensitivity comparison with published mAbs

Pesticide	Clone name	Isotype	IC_{50} (ng/ml)	
			This work	Literature[a]
Triazophos	THHe-QA2-8C10	IgG1, *lambda*	0.91	2.54 (Jin *et al*., 2008)
Parathion	PA0204-QA1-7C2	IgG1, *kappa*	2.46	2.94 (Wang *et al*., 2009); 7.06 (Li *et al*., 2015)
Chlorpyrifos	CHBu-QA2-13C7	IgG1, *kappa*	3.72	24 (Kim *et al*., 2011); 3.5 (around 10 nmol/L) (Manclús *et al*., 1996)
Fenpropathrin	NC-S-2-QA2-1C3	IgG2a, *kappa*	6.22	14.03 (Chen *et al*., 2014)

[a] The mAbs for comparison were derived from immunizing haptens with the same or very similar structures, and sensitivities were tested by competitive indirect ELISAs

The specificity of the mAbs was measured by cross-reactions with related pesticide analogues. As seen from Fig. 4, these new mAbs were generally specific to the target analytes, except that mAbs for parathion and chlorpyrifos had around 20%–25% cross-reactivity to methyl-parathion and methyl-chlorpyrifos, respectively. As shown in Fig. 5, mAb for fenpropathrin exhibited around 4%–15% cross-reactivity to cypermethrin, deltamethrin, and cyhalothrin. These minor cross-reactions seemed difficult to avoid on the basis of their similar structures, which were also observed in previous work (Wang *et al*., 2009; Liu *et al*., 2011; Chen *et al*., 2014). Certainly, cross-reactivity data herein may vary somewhat from values obtained in future applications, because of different assay formats, reaction systems, and so on.

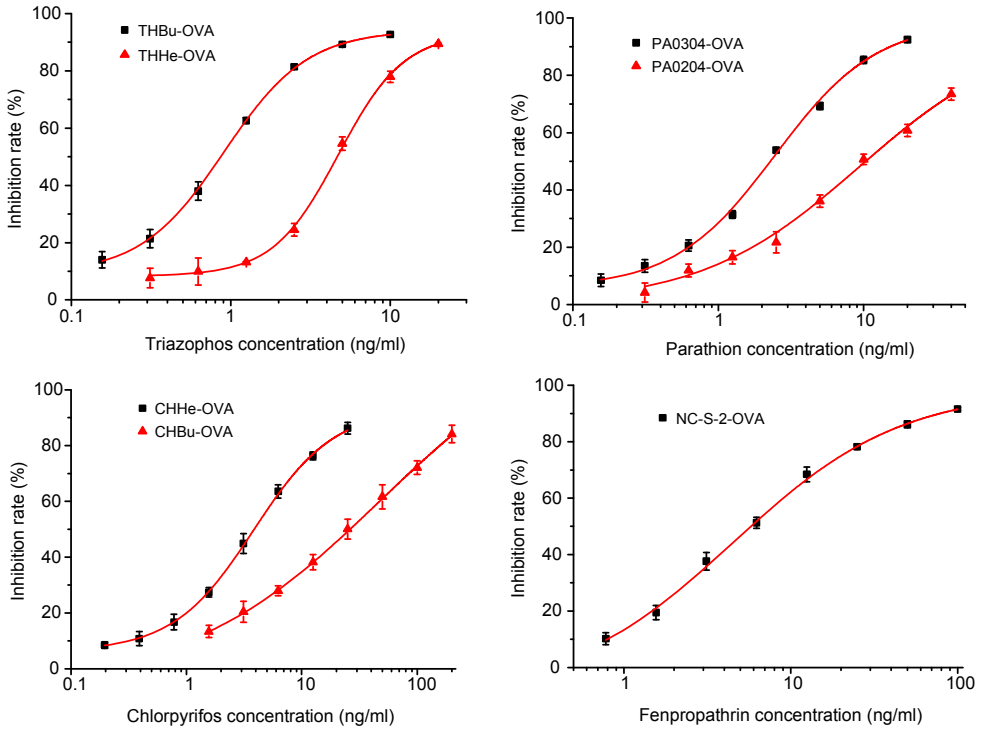

Fig. 3 Standard curves of pesticides by indirect competitive ELISAs based on related coating antigens
Data are expressed as mean±SD, with n=6

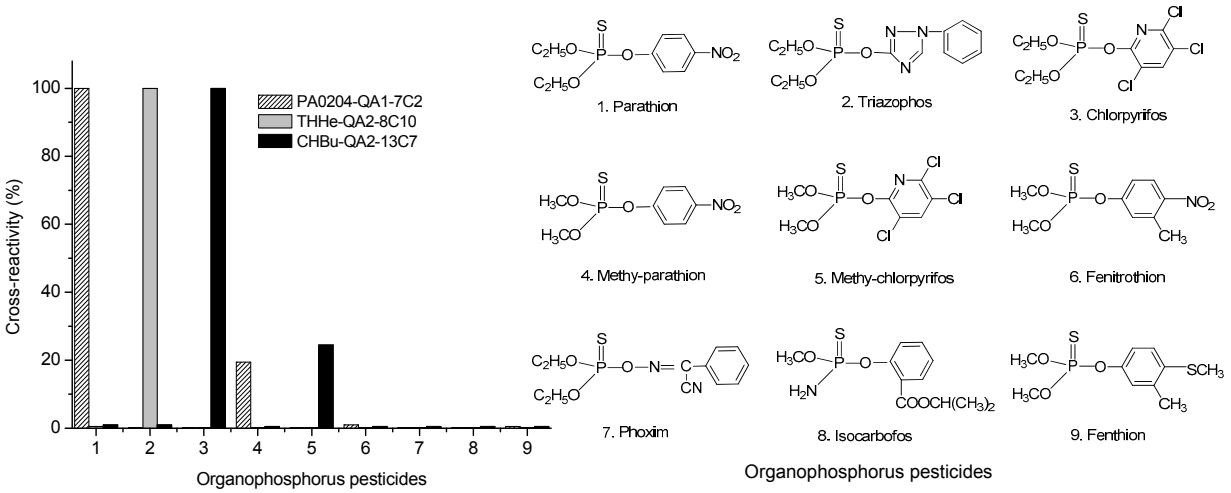

Fig. 4 Cross-reactivity study of three new mAbs to some organophosphorus pesticides

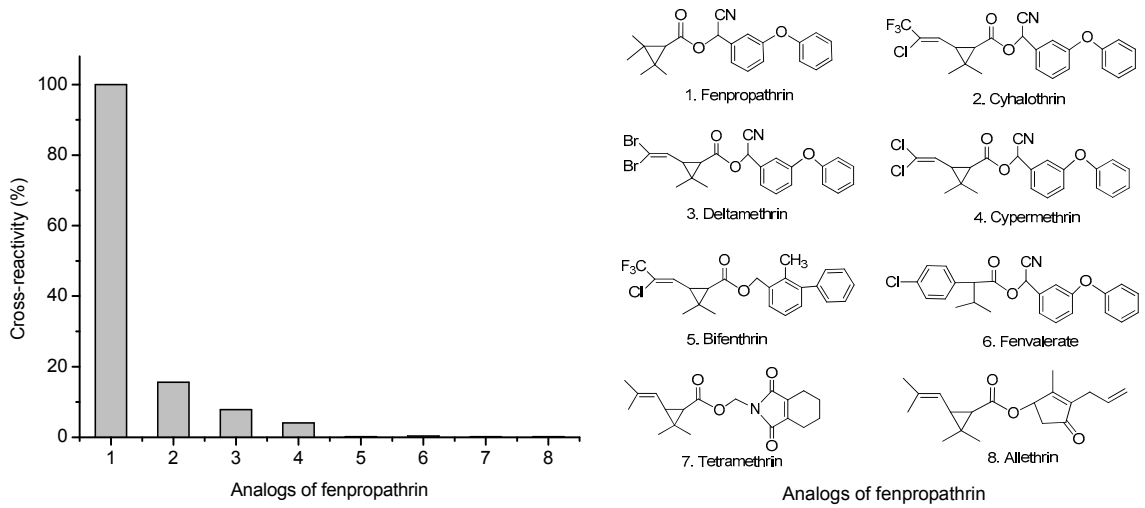

Fig. 5 Cross-reactivity results of mAb NC-S-2-QA2-1C3 to other pyrethroid pesticides

3.5 Development of mouse antibody for okadaic acid

Since OA is a kind of marine biotoxin and its standard is expensive, the dosage for effective immunization is usually chosen to be as small as possible. Having achieved satisfactory performance in pesticide trials, we directly used QA adjuvant for OA-BCP injection to check if it could also help provoke high-affinity antibodies toward OA with fewer inoculations over a relatively short duration.

One week after the first booster injection (Day 28), mouse antisera were collected and characterized by non-competitive indirect ELISAs coated with OA-BSA at the concentration of 5 μg/ml. The titers ranged from 1/4000 to 1/16000 for three OA-BCP mice, suggesting pretty quick and good immune responses. After cell fusion and hybridoma selection, a most sensitive and stable clone named OA-QA1-1H10 was finally acquired, which secreted mAb against OA with IC_{50} of 0.36 ng/ml (Fig. 6). The sensitivity profile was much better than those of some similarly reported mAbs, which were produced from traditional Freund's (IC_{50} 4.40 ng/ml (Lu *et al*., 2012)) and other kinds of adjuvants (IC_{50} 4.80 ng/ml (Stewart *et al*., 2009)). Also, it was very close to that from the recent work showing IC_{50} of 0.58 ng/ml based on the competitive indirect ELISA (Liu *et al*., 2014), where Freund's complete adjuvant was used only once for the primary inoculation during the whole immunization period. Additionally, in the three previous studies, it took 4–6 inoculations and the total immunogen dose of 240–300 μg per mouse.

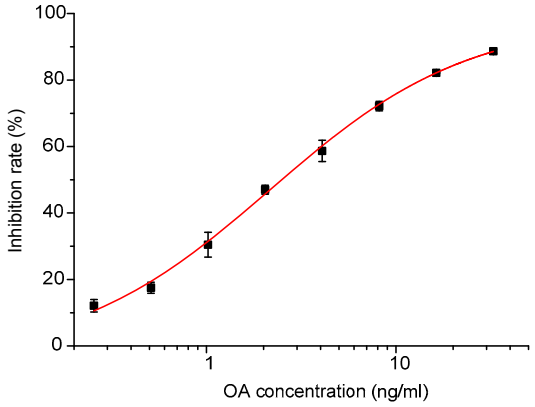

Fig. 6 A standard curve for okadaic acid (OA) based on the clone OA-QA1-1H10 and 1 μg/ml of OA-BSA-coated ELISA

Data are expressed as mean±SD, with n=6

The present results demonstrate that QA adjuvant could promote strong immune responses for OA within two immunizations (over less than one month). It seemed to be relatively time-saving, labor-saving, and cost-effective, and particularly may be suitable for the efficient development of antibodies against expensive and toxic chemicals like biotoxins. Even so, we still expended a total 150 μg of immunogen per mouse in this work. Thus, a lower dosage for immunization should be applied in future studies, in view of the biotoxicity of marine toxins and their high costs. Nonetheless, undoubtedly, there is no single adjuvant that is right for all situations. The study of the application of QA to other types of antigens is on-going.

4 Conclusions

An easy-to-use QA adjuvant was employed and investigated for producing mAbs toward different kinds of low-molecular-weight compounds. Results indicated that under the recommended immunization strategy, the antiserum titers elicited from QA adjuvant were relatively lower than those from Freund's adjuvants with the same dose of immunogen, while the fusion efficiency for immune-positive clones was still satisfactory in the administration of QA. As a result, five kinds of newly developed mAbs were obtained with high sensitivities to the corresponding target analytes, which can be applied to the trace-analysis of these contaminants in food and environmental samples. Additionally, by using QA adjuvant, it only required 2–3 immunizations before cell fusion, which fairly shortens the conventional immunization period for generating hapten-specific mAbs. Therefore, the new water-soluble adjuvant could be used for rapidly producing effective mAbs against small-molecule contaminants, although only part of its advantages as claimed by the manufacturer was confirmed in the current work. Further comparison studies with other adjuvants should be carried out.

Compliance with ethics guidelines

Rui LIU, Ying LIU, Mei-jing LAN, Niusha TAHERI, Jing-li CHENG, Yi-rong GUO, and Guo-nian ZHU declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：一种水溶性免疫佐剂应用于小分子化合物单克隆抗体制备的效果评价

目　的：评价一种新型水溶性免疫佐剂在制备小分子化合物单克隆抗体（单抗）中的应用效果。

创新点：采用一种新型水溶性免疫佐剂，快速制备了分别针对 4 种农药和 1 种海洋毒素的高亲合力单抗，且其性能与以往采用弗氏佐剂获得的相应单抗非常接近。

方　法：本研究以 4 种农药和 1 种海洋毒素为目标分析物，采用一种新型水溶性佐剂与小分子抗原相互混合，直接对 Balb/c 小鼠进行小腿肌肉注射免疫。经过 2~3 次免疫后进行腹腔注射常规末次免疫，细胞融合后筛选出了相应的杂交瘤细胞株，制备并鉴定了针对上述半抗原小分子的特异性单抗。

结　论：采用该水溶性免疫佐剂制备抗原注射溶液无需乳化过程，免疫操作简便，且对小鼠负面效应较小；虽然该处理小鼠产生的抗血清效价并没有像常规弗氏免疫佐剂带来的效价值高，但细胞融合后筛选得到有效细胞株的概率尚可；获得的针对目标分析物的单抗在灵敏度和特异性等方面都接近于以往采用弗氏佐剂获得的单抗。试验结果表明，该水溶性免疫佐剂适用于针对小分子化合物单抗的高效制备。

关键词：水溶性免疫佐剂；单克隆抗体；半抗原特异性；高亲合力