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(57) Abstract

A method for producing broad specificity, generic, antibodies having a specific binding affinity for two or more of a class of compounds demonstrating a common mode of biological activity (e.g. organophosphate pesticides) comprising selecting a receptor or enzyme through which the class of compounds exert the biological activity, raising intermediate monoclonal antibodies against the recognition site of the receptor or enzyme, inoculating an animal with these iMABs or fragments thereof and recovering the generic antibodies from a body fluid or tissues of the animal. Also disclosed are generic polysera, generic antibodies, and cell lines for producing them, and also methods and kits for screening a sample for the presence or absence of compounds demonstrating a common mode of activity using generic antibodies.

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BROAD SPECIFICITY ANTIBODIES

The present invention relates to antibodies having a broad specificity. The invention further relates to methods of raising such antibodies, and to processes and kits employing them.

There is a requirement for broad specificity, generic, antibodies i.e. antibodies which are able to specifically bind to several members of a class of related compounds. Such antibodies have utility in areas of analysis of groups of compounds which exert similar activities by means of a particular binding ability e.g. drugs, pesticides, mycotoxins, agonists, antagonists, vitamins etc. The antibodies can be used in screening assays to detect the presence of known or novel compounds having the particular binding ability, and also to extract the compounds (the immunoaffinity approach).

In areas of analysis aimed at detecting groups of compounds demonstrating a common mode of action e.g. organophosphate pesticides, conventional antibodies are often too specific to be able to recognise more than a single member of the group. Thus generic immunoassays must employ several different antibodies in parallel or series.

WO 94/27149 discloses a strategy for raising antibodies which are specifically directed to bind two or more specified pesticidal compounds. The disclosed method employs a hapten-protein conjugate having a particular phosphate nucleus; antibodies raised against the conjugate have binding ability for several organophosphates. This methodology requires chemical synthesis of the hapten structure, and can not be readily extended to cover other groups of compounds e.g. veterinary drugs, which may have less structural similarity within the group.

The present inventors have now provided a novel strategy for producing generic antibodies which does not have the drawbacks of this prior art.

In a first aspect of the invention there is disclosed a method for producing generic antibodies having a specific binding affinity

for two or more of a class of compounds demonstrating a common mode of biological activity comprising selecting a receptor or enzyme through which the class of compounds exert the biological activity, raising intermediate monoclonal antibodies (iMABs) against the recognition site of the receptor or enzyme, inoculating an animal with these iMABs or fragments thereof and recovering the generic antibodies from a body fluid or tissue of the animal

The specific binding affinity is preferably such that the two or more compounds are bound with relative affinities in the range of 1 to 10^4, more preferably between 1 and 10^3, and ideally between 1 and 10^2, as measured by standard binding assays. In this way the generic antibodies can be used to detect or extract the compounds from materials even when the compounds are present in widely varying proportions. Preferably the generic antibodies detect at least four compounds from the class.

It is well known in the art that many classes of compounds demonstrate a common mode of activity because they contain a common structural motif which interacts with a complementary 'recognition' site within a receptor (i.e. the binding site) or enzyme (i.e. the active site). This interaction generally triggers a structural change within the receptor or enzyme which triggers the activity in question. The term 'biological activity' as used herein is used in a broad sense, and is intended to include *inter alia* inhibition of the receptor or enzyme (as in the case of organophosphates) or modification (e.g. hydrolysis) of the compound used.

Once the class of compounds against which the generic antibody is to be raised has been selected, the receptor or enzyme through which the class of compounds exert the biological activity can be selected in accordance with information available in the art. This may one for which the compounds are a natural substrate or agonist, alternatively it may be one for which the compounds are an artificial substrate or antagonist, or an inhibitor.

Preferably the receptor or enzyme has a recognition site which is readily accessible to an antibody i.e. which is not concealed within a cleft. Alternatively the antibody could be raised against a peptide corresponding to the epitopic features of the recognition site.

Methods of raising MABs against a particular antigen have long been known in the art (see e.g. Kohler and Milstein (1975) Nature 256: 495-497). In the present invention it is essential that the iMABs are directed against the recognition site, and not some other portion of the antigen. This can be deduced, *inter alia*, by testing hybridoma cell lines to see if the iMABs produced therefrom have inhibitory activity against the enzyme or receptor in question, and whether the binding of the iMABs to the enzyme or receptor is itself inhibited by the presence of the compounds themselves - both of these phenomena are indicative of iMABs which bind the binding/active site directly.

Once selected the iMABs, or binding-site fragments thereof, are themselves used as immunogens. The immunogens can be in either unconjugated or conjugated (e.g. with BSA) form. Antibodies raised against the binding site of the iMABs will themselves have a binding site which mimics the original enzyme or receptor. Thus these generic antibodies will have a specific binding affinity for the compounds of interest, and can be selected or isolated by immunoaffinity techniques using one or more of the compounds e.g. in immobilised form.

The generic antibodies raised by the method above will be polyclonal. Thus in a second aspect of the invention there is provided a method for producing cells capable of producing generic MABs (gMABs) having a specific binding affinity for two or more of a class of compounds demonstrating a common mode of biological activity comprising a method as described above but wherein, rather then recovering antibodies from a body fluid of the animal, antibody-producing cells are removed from the animal and are used to create hybridomas using methodology well known to those skilled in the art. Alternatively, the DNA from the antibody-producing cells is used to transfect other cells in which it can be

expressed. The hybridomas or transfected cells can be selected by use of immunoaffinity techniques which detect the gMABs produced therefrom.

In a third aspect of the invention there is provided a method for producing gMABs having a specific binding affinity for two or more of a class compounds demonstrating a common mode of activity comprising culturing the cells produced as above and isolating gMABs from the culture medium.

The term 'generic antibodies' as used hereinafter is intended to include both polyclonal- and monoclonal- generic antibodies.

The materials produced by the methods described above i.e. the generic polysera, generic antibodies, and the cell lines all constitute a further aspect of the present invention. Likewise, such materials which have been further manipulated (e.g. by use of recombinant DNA technology) to improve their stability or specificity are also embraced by the present invention. Techniques for manipulating the antibody binding site by accessing the genes which encode the antibody variable regions are now known in the art. For instance, following sequencing and modelling of the binding site, changes can be made in selected bases by site-directed mutagenesis to alter the amino acids involved in binding the compounds. Alternatively, random mutagenesis can be performed on the antibody gene and the resultant antibodies can be screened for improved binding characteristics.

The invention further makes available a method of screening a sample for the presence or absence of compounds demonstrating a common mode of activity comprising contacting the sample with generic antibodies produced as described above under conditions sufficient to permit the formation of immune complexes; detecting the formation of immune complexes; and relating the presence or absence of immune complexes to the presence or absence of the compounds.

The invention further makes available a method for extracting compounds demonstrating a common mode of activity from a sample comprising a screening method as claimed above wherein any immune

complexes formed are treated such as to dissociate the compounds therefrom e.g. using acetone.

The invention further makes available kits for use in the methods above comprising one or more of the materials (i.e the generic polysera, the generic antibodies, or the cell lines) described above. Preferably the kits comprise gMABs plus means for detecting the formation of immune complexes such as are well known to those skilled in the art e.g. ELISA. Detection may take the form of a displacement assay wherein the presence of compounds displaces a labelled marker (e.g. the immunogen used to raise the antibody, or a labelled compound of the type bound by the antibody) from the generic antibody binding site. The displacement of this marker can then be related to the presence of the compounds.

Kits could take the form of dip-sticks or card assays such as can be devised by the skilled addressee in the light of the present disclosure without undue burden.

The methods, materials and kits described above are particularly advantageous when used to detect or extract organophosphates. Organophosphates all contain a central P(S) or P(O) entity and are employed as pesticides and nerve-agents. Many exert their properties via interaction with the active site of hydrolases e.g. acetylcholinesterase, serine proteases, parathion hydrolase and cutinase, thus the active site constitutes a recognition site for these compounds. Although enzymes having a recognition site could themselves be used for organophosphate screening, enzymes have the disadvantages that they are generally less robust, more expensive, and less able to be manipulated then MABs. These enzymes are, however, candidates for use in raising the iMABs employed in the present invention.

Organophosphates are frequently employed as pesticides on food crops, common agents being chlorpyrifos, malathion, chlorfenvinphos and tetrachlorfenvinphos. It is important to test foodstuffs derived from treated plants for organophosphate residues to ensure that they do not breach legal requirements. A test based around the generic MABs of the present invention provides an innovative, robust, cheap and rapid solution to the

problem of mass screening of food samples for the presence of pesticide residues, and would enable rapid elimination of negative samples from surveillance programs. Positive samples could be further tested to establish the precise nature of the contaminants present.

Thus in one preferred embodiment, the invention makes available a method of screening a sample for organophosphate compounds comprising a method of screening a sample as described above wherein the iMABs are raised against an enzyme or receptor which binds or is inhibited by organophosphates e.g. a serine protease, but most preferably cutinase.

Depending on the specificity of the generic antibody, the method may be limited to either P(O) or P(S) based organophosphates. Preferably, however, the original enzyme or receptor is selected such as to generate generic antibodies having an affinity for both types of organophosphate.

Preferably the sample to be screened is a food item. However, the screening method could also be used for the purpose of screening samples for novel compounds having organophosphate type activities e.g. screening environmental samples for new lead compounds for use as pesticides.

Thus the invention makes available methods, materials and kits with utility in the field of screening for, or extracting, compounds demonstrating a common mode of activity.

Other particularly advantageous applications of the present invention employ the ability of gMABs to bind a wide variety of human or veterinary drugs. For instance ß-lactam antibiotics which can be detected using antibodies based around the ß-lactamase active site.

Some methods and materials of the present invention will now be described, by way of illustration only, through reference to the following example and figure. Other embodiments falling within the scope of the invention will occur to those skilled in the art in the light of these.

FIGURE

Fig 1 shows displacement of gMAB 1507 from an immobilised pesticide conjugate by four different pesticides.

EXAMPLE 1 - A GENERIC MAB HAVING SPECIFIC BINDING AFFINITY FOR ORGANOPHOSPHATE PESTICIDES

1) Immunisation protocol

Mice or rabbits were injected subcutaneously on the back with immunogen dispersed in saline:Freunds adjuvant (1:1, v:v). Incomplete Freunds adjuvant was used, except for the first injection of each animal where complete Freunds adjuvant was substituted. Rabbits were bled twice from the marginal ear vein, and mice were bled once from the tail, 10-14 days after injection.

2) Preparation of MABs to cutinase

Murine MABs were raised to cutinase using the methods described by Galfr and Milstein (1981) Methods in Enzymology 73, 3-46. Spleenocytes were removed from the mouse 4 days after immunisation and fused with myeloma cells in a ratio of between 3:1 and 10:1 respectively in the presence of polyethylene glycol. After 10 days fusion plates were screened by ELISA and positive binding wells expanded for rescreening. Suitable cell lines were cloned 3 times by the limiting dilution method, and wells producing antibody identified by ELISA. The MABs were then grown up in quantity in flasks, in Optimem medium containing 1-4% foetal calf serum (FCS).

3) Purification of MABs

MABs were purified from culture supernatant by initial concentration using a Minitan concentrator fitted with 30 kDa cut-off membranes (Millipore Ltd, Watford, U.K.). This process reduced the volume from 11 to 60 ml. The solution was then taken to 60% saturation at 4°C with ammonium sulphate and the protein pelleted by centrifugation at 10,000 rpm for 30 mins at 4°C. The protein was resuspended in double glass-distilled deionized water and then buffer-exchanged into 1.5 M-glycine/NaOH, pH 8.9,

containing 3M-NaCl using Sephadex PD10 column (Pharmacia Ltd, Milton Keynes, U.K.). Antibodies were purified by affinity chromatography using a Protein A-FPLC column (Pharmacia Ltd) dialysed against 10 mM-ammonium hydrogen carbonate, and then freeze dried.

4) Screening iMAB cell lines for cutinase-inhibiting activity

GENERAL CUTINASE ASSAY: The assay for cutinase activity described by Kolattukudy et al (1981) Methods in Enzymology 71, 652-664 using p-nitrophenylbutyrate as substrate was adapted for microtitration plates. The substrate was prepared as described except that the solution was clarified using an ultrasonic bath. The basic assay was as follows: 100 µl of 0.1 M-phosphate-buffered saline, pH 7.4, containing 0.05% (v/v) Tween 20 (PBST) was added to 50 µl/well of cutinase (40 ng ml^{-1} in PBST) in the appropriate wells of a microtitration plate, followed by 50 µl (50µM) of p-nitrophenylbutyrate. The rate of increase in absorbance at 410 nm due to the formation of p-nitrophenol at room temperature was measured every 15 seconds for 7.5 mins using a Dynatech MR5000 plate reader (Dynatech Laboratories Ltd, Billinghurst, U.K.). Control reactions measuring substrate autohydrolysis were included on the microtitratrion plate by replacing the enzyme in these wells with an equivalent volume of buffer. Reaction progress curves were depicted for each microtitration plate well using the RMS programme supplied with the MR5000 plate reader. Initial velocities of the enzymic reactions were calculated by linear regression.

Initial experiments showed that 2 ng of cutinase in appropriate buffers produced a suitable rate of substrate hydrolysis. This amount of enzyme and the substrate concentrations indicated above were used to assess hybridoma cell lines for production of appropriate iMABS able to inhibit enzyme activity. These assays were performed by transferring 50 µl from 96-well microtitration fusion plates to sterile 96-well microtitration plates containing PBST (50 µl) and cutinase (50 µl; 2 ng). Control reactions contained cell culture medium (50 µl), PBST (50 1) and enzyme (50

µl). Reactions were initiated by the addition of 50µl (50 µM) substrate.

From 360 seeded fusion plate wells 18 were shown to contain hybridomas secreting antibody that inhibited cutinase activity. Subsequent cloning of these hybridomas established 6 stable cell lines. The antibodies from these cell lines, designated iMABs IFRN 1901-1906, were all shown to be of the IgG1 isotype and were purified by Protein A affinity chromatography. Differences were seen in the degree by which cutinase activity could be inhibited by purified antibodies. iMABs 1901, 1902, 1904 and 1905 (40 ng) inhibited cutinase activity by 80 to 90%. At the same antibody concentration iMABs 1903 and 1906 inhibited the enzyme 58 and 30% respectively.

5) Kinetic study of iMAB-cutinase interaction

To evaluate the effect on kinetic parameters Km and Vmax, and so determine the mode of inhibition of cutinase by the iMABs, substrate concentration was varied from 50-500 µM and antibody concentration from 0-40ng. Assays were as described above except that the assay temperature was maintained at 25°C. Data was analysed by the method of Wilkinson (1961) Biochem J. 80, 324-332.

Kinetic analyses (Table 1) showed that for all the enzyme-inhibitory antibodies, Km remained largely unchanged whereas Vmax was much decreased, reflecting the high affinity of the antibodies for the enzymes resulting in a reduction in the number of active enzyme molecules able to hydrolyse substrate. This indicated that it would be difficult to determine, by kinetic studies, whether the epitopes recognised by the MABs were located at or near the enzyme active site, or if inhibition resulted from antibody-induced conformational change in the enzyme molecule.

6) Screening iMABs for binding to pesticide-inhibited cutinase

The affinity of antibodies 1901-1906 for cutinase that had been inhibited by the organophosphate pesticides methyl paraoxon and chlorfenvinphos was studied.

Table 1 : 40 ng of antibody used

ANTIBODY (cutinase only)	Km (mM) 0.66±0.04	Vmax (min-1) 0.19±0.01
1901	0.46±0.06	0.05±0.01
1902	0.78±0.13	0.05±0.01
1903	0.76±0.10	0.04±0.01
1904	0.36±0.05	0.06±0.01
1905	0.65±0.03	0.05±0.01
1906	0.70±0.10	0.05±0.01

Cutinase (1 mg) was incubated with chlorfenvinphos and methyl paraoxon (1.8 and 1.1 mM respectively) at room temperature in PBST. Samples were desalted on a Sephadex PD-10 column equilibrated with PBST. These samples together with uninhibited cutinase were used to coat sections of the same microtitration plates at 1g ml^{-1} in 0.05M-carbonate/bicarbonate buffer, pH 9.6. Purified MABs diluted PBST (1-1000 ng ml^{-1}) and added to the microtitration plates which were then incubated at 37°C for 2 hrs. After being washed 5 times with PBST, anti-mouse IgG horseradish peroxidase (HRP) conjugate (Dako Ltd, High Wycombe, U.K.) diluted 1:15000, v/v, with PBST was added (200 l $well^{-1}$), and the plates incubated for 2 hrs at 37°C. Again the plates were washed 5 times with PBST, then substrate was added (200l $well^{-1}$) and absorbance readings were measured on a Titertek Multiscan plate reader (Flow Laboratories, Irvine, U.K.) at 450 nm.

This test showed that all the antibodies had a reduced affinity for pesticide-inhibited cutinase. Table 2 gives the results for iMAB 1901. These results alone do not indicate whether the reduced binding arises from a conformational change induced in the enzyme following pesticide inhibition. However X-ray crystallographic analysis of an ethyl paraoxon-cutinase complex by

Martinez et al (1994) Biochemistry 33, 83-89 shows that there is no conformational change in cutinase following inhibition by an organophosphate pesticide. This observation, together with those reported above, strongly suggest that iMABs 1901-1906 bind at, or close to, the active site of cutinase, and that these antibodies can act as pesticide mimics and be used to generate gMABs that would bind to a broad spectrum of organophosphate pesticides.

Table 2

iMAB (ml-1) concentration	Enzyme (OD450 nm) alone	Chlorfenvinphos inhibited	Methyl paraoxon inhibited
10 g	1.40	0.33	0.23
1 g	1.51	0.20	0.12
100 ng	1.40	0.13	0.09
10 ng	1.24	0.08	0.05
1 ng	1.12	0.06	0.06

7) Screening iMABs for binding to reduced-denatured cutinase

To test whether the iMABs recognised enzyme conformation, the binding of the antibodies to denatured cutinase was examined by SDS electrophoresis and protein transfer to nitrocellulose membranes. If the antibodies recognised discontinuous epitopes of the enzyme folded in its native state, then they should not bind to the denatured enzyme.

Purified anti-cutinase MABs were screened for binding to reduced-denatured cutinase by electrophoretic techniques. Cutinase was boiled for 15 minutes with 25% (w/v) sodium dodecyl sulphate (SDS) in Tris/HCl buffer (pH 8.0). The reduced samples were then separated by electrophoresis using a Pharmacia Phast Gel Electrophoresis System (Pharmacia Ltd, St. Albans, U.K.) and SDS-polyacrylamide gels. Subsequently protein in the SDS gel was transferred to nitrocellulose membranes (Sartorius Ltd,Epsom,U.K)

again using the Phast Gel System. Transfer took 30 mins after which the nitrocellulose membranes were incubated for 12 hrs at 4°C with 0.2 M, pH 7.4 phosphate buffered saline (PBS) containing 5% (w/v) skimmed milk powder to block remaining protein binding sites on the nitrocellulose membrane. After blocking, the membranes were extensively washed with PBST then cut into 6 strips which were each incubated at 37°C for 1 hr with separate solutions of iMABs 1901-1906. The membranes were again extensively washed and then incubated at 37°C for 1.0 hr with anti-mouse IgG-alkaline phosphatase conjugate (Sigma Chem Co. Ltd, Poole, U.K.). After washing the membranes with PBST, they were incubated with alkaline phosphatase substrate consisting of 5-bromo-4-chloro-3-inodolyl phosphate, magnesium chloride and nitrobluetetrazolium in ethanolamine buffer, pH 9.8. This substrate mixture gives a coloured precipitate following reaction on the nitrocellulose membrane at the region where the anti-mouse enzyme conjugate has bound.

In this experiment all the iMABs bound to denatured enzyme indicating that they recognised a continuous epitope that could encompass the enzyme active site.

8) iMABs immunogen synthesis

The types of iMAB conjugate used as immunogens to raise generic antibodies are shown in Table 3.

MAGNETIC BEAD CONJUGATES: The hydroxyl groups of polysaccharide coated magnetic beads (Dynal, New Ferry, U.K.) were activated using 2-fluoro-1-methyl pyridinium toluene-4-sulphonate (FMP). iMAB 1904 in sodium hydrogen carbonate (0.2M) was mixed with activated magnetic beads from the supernatant which was stored frozen. Beads were resuspended in 0.1 M, pH 8.0 Tris/HCl buffer to block any remaining FMP-activated hydroxyl groups. Beads were then extensively washed with 20 mM, pH 7.4 phosphate buffer containing 0.5M NaCl (PBS) and stored frozen. The effectiveness of the conjugation was assessed by testing the stored supernatant for inhibition of cutinase activity. This test was negative, indicating complete conjugation of antibody to beads.

Table 3

iMAB	Carrier
1901	None
1902	None
1903	BSA
1904	Magnetic beads
1905	BSA
1906	Haemocyanin

PROTEIN CONJUGATES: iMABs 1903, 1905 and 1906 were conjugated to bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH) using 1.0% (v/v) glutaraldehyde in PBS. A bifunctional photoactivatable compound, p-azidobenzoyl hydrazide (Pierce Ltd, Chester, U.K.) was used to conjugate 1905, via its sugar residues, to the amino groups of BSA and KLH. For both coupling methods, conjugated antibody was separated from unconjugated material using Sephadex G200. Conjugated antibody was shown to be active using the cutinase inhibition assay.

9) Immunisation protocol

This was as discussed at 1). Animals were immunised with conjugated and unconjugated anti-cutinase antibodies.

10) Screening polyclonal sera for production of generic antibodies using pesticide-coated microtitration plates

To select for generic antibodies that would recognise organophosphate pesticides, immune sera were screened against a trans-4-phosphono-2-butenic acid diethyl ester (TPB) protein conjugate immobilised on a microtitre plate. TPB possesses two diethyl groups attached to a phosphate moiety, a structure possessed by a large number of organophosphate pesticides.

TPB was conjugated to human serum albumin (HSA) and bovine thyroglobulin (BTG) by making use of carbodiimide activation of the TPB carboxylic acid group to form an O-acylurea and subsequent amide bond formation with free amino groups on the two proteins.

Mouse tail bleeds were serially diluted 1:100, 1:500 and 1:1000 (v:v) with PBST and 50 l well-1 of each dilution added to 50 µl of PBST in microtitration plates coated with TPB-BTG. Plates were incubated at 37°C for 1.5 hrs, washed with PBST and then 100 µl $well^{-1}$ anti-mouse IgG HRP conjugate diluted 1:1000 (v:v) in PBST added. Plates were again washed after 1.5 hrs incubation at 37°C. and HRP substrate (Cambridge Life Sciences Ltd, Ely, U.K.) added. Plates were incubated at 37°C for 10 mins at which point the chromogenic reaction was stopped by adding 50 µl $well^{-1}$ of 2M H_2SO_4. Optical densities of the wells were then determined.

Polyclonal sera were also tested for the ability of a mixture of pesticides (chlorpyriphos, chlorfenvinphos, carbophenothion and ethyl paraoxon; Promochem, Welwyn Garden City, U.K.) to inhibit antisera binding to microtitration plates coated with TPB-BTG. In these assays the pesticides were mixed in equal amounts to give a total concentration range of 45 ng to 45 g ml-1. The pesticide solutions were added to microtitration plates coated with TPB-BTG and the assay continued as described above.

Polyclonal sera from mice and rabbits immunised with iMABs 1901-1906 were found to bind to TPB conjugate. However it was only possible to inhibit the binding of four of the antisera from mice, but none of the sera from rabbit with a mixture of pesticides consisting of carbophenothion, chlorpyrifos, chlorfenvinphos and ethyl paraoxon. These antisera were produced by immunisation with 1906-hemocyanin, 1905-BSA, 1901 (unconjugated) or 1902 (unconjugated) and were inhibited from binding to TPB-BTG by the mixture by between 36-45%.

11) Preparation and purification of gMABS

This was carried using the methods described at 2) and 3).

12) Screening hybridoma cell lines for production of gMABs using pesticide-coated microtitration plates

Hybridomas growing in the wells of microtitration fusion plates were screened for the production of generic antibodies which could bind to TPB-BTG and CPT-B-lactoglobulin and for inhibition of this binding by the pesticides carbophenothion, chlorfenvinphos and ethyl paraoxon. Carbophenothion and chlorfenvinphos were conjugated to B-lactoblobulin as follows. Thiol groups were introduced onto B-lactoglobulin by reacting this protein with s-acetylmercaptosuccinic anhydride. Thiolated protein was then conjugated to each pesticide via displacement reaction between the chlorine groups of the pesticide and the thiol groups of the protein by mixing in 0.1 M, pH 8.0 phosphate buffer at room temperature for 12 hrs. The conjugation of carbophenothion to protein was demonstrated by calorimetric assay using palladium chloride. This reacts with the P=S bond of the pesticide to give an orange/yellow-brown solution.

Supernatants from the cells were transferred, using a Biomek 1000 Automated laboratory Workstation (Beckman Ltd, High Wycombe, U.K.) in duplicate to wells of a microtitration plate coated with the pesticide conjugates. One set of wells contained PBST only (in order to assess binding to the pesticide conjugate) and one set contained pesticide mixture (45 µg ml-1)(in order to assess inhibition of binding). The assay was carried out as described above.

Initial screening of the fusion wells detected several MABs binding to pesticide-protein coated plates which were displaced by the mixture of pesticides used to test the polyclonal sera. After expansion and cloning only one cell-line remained stable, producing a gMAB which bound strongly to TPB, chlorfenvinphos, carbophenothion and chlorpyrifos immobilised on the plate, but not to a parathion-BSA conjugate. This was designated gMAB 1507 (produced by immunisation with iMAB 1901.)

To test gMAB 1507 for inhibition of binding to an immobilised pesticide-conjugate by pesticides in solution the culture supernatant containing the MAB was diluted 5-fold. The pesticides

tested are shown in Table 4. Four pesticides demonstrated displacement of antibody in the assay and the standard curves over the range 5 pg - 5 mg/well are shown in Fig. 1. Demeton-S-methyl displaces at 5 µg/well and tetrachlorfenvinphos at 5 µg/well, however, displacement was greatest for ethyl paraoxon and chlorfenvinphos with a sensitivity in each case of 500 pg/well. The results were similar whichever pesticide conjugate was used on the plate. Further experiments were conducted to show that gMAB 1507 demonstrated the expected characteristics.

Table 4: Pesticides tested for inhibition of binding of gMAB 1507 to a chlorfenvinphos-conjugate in ELISA

Pesticide	Least amount of pesticide to give significant inhibition ($well^{-1}$)
Chlorfenvinphos	500 pg
Ethyl paraoxon	500 pg
Tetrachlorfenvinphos	5 µg
Demeton-S-Methyl	5 µg

The following pesticides were also tested but showed no inhibition of binding: azamethiphos, edifenphos, propetamphos, phenthoate, menvinphos, methamidiphos, sulfotep, dichrotophos, valmidothion, fosamine ammonium, fenamiphos, monocrotophos, cyanofenphos, dichlorvos, malathion, parathion ethyl.

13) Testing displacement of gMAB 1507 from pesticide-coated microtitration plates by iMABs

In addition to displacement by pesticide, gMAB 1507 should also be displaced from binding to immobilised pesticide with iMAB 1901. Table 5 shows the result of a chequerboard ELISA where iMAB 1901 and gMAB 1507 were added together at differing dilutions onto a CPT-B-lactoglobulin coated plate. The percent binding of gMAB

1507 in the presence of increasing concentrations of iMAB 1901 compared to the binding of gMAB 1507 alone show that it is prevented from binding to the pesticide by iMAB 1901. It was also shown that other cutinase-inhibiting iMABs could displace gMAB 1507, but to a lesser extent.

Table 5: Displacement of gMAB 1507 by iMAB 1901 from a CPT-B-lactoglobulin plate

1507 dilution	1901 dilution		
	1:0	1:1	1:2
1:0	13	67	91
1:1	31	52	85
1:3	27	59	95
1:7	24	52	80
1:15	17	41	79

14) Columns for extracting pesticides from a sample using gMAB 1507

Affinity columns in which - gMAB 1507 was covalently linked to a solid phase support were prepared by two methods.

a) Protein A-Agarose: Immunopure Protein A (1 ml, Pierce) was washed in Tris buffer and allowed to react with 11 mg of purified MAB. Unbound sites were deactivated with triethanolamine. Although Protein A has a strong affinity for mouse IgG there is no covalent link between them and the MAB can be disassociated, for instance with high pH or high salt concentration. To avoid this, the Protein A and IgG were cross linked by reaction with 15 mM pimelidate followed by ethanolamine to block unreacted sites and washing in borate buffer.

b) CNBr-Sepharose: CNBr-activated Sepharose (0.3g, Pharmacia) was allowed to swell and repeatedly washed on a scintered funnel with dilute HCl before washing in carbonate buffer. The gel was reacted with 11 mg of purified MAB on a rotating wheel for 2 hrs and subsequently washed with carbonate buffer. After standing overnight in Tris buffer the gel was washed 5 times alternately with acetate and Tris buffers of pH 4.0 and 8.0 respectively.

The MAB-conjugated gels were stored in phosphate-biffered saline (PBS) at 4°C until required. The columns were prepared by filling a 1 ml syringe with 0.2 ml of one of the gels, and washing with 3 x 5 ml PBS.

In use, the sample is prepared in 1 ml PBS and applied to a column. The column is washed with PBS (3ml) followed by 2 x 1 ml of acetone to elute any bound pesticide, before being re-equilibrated in PBS.

The eluted pesticide is then extracted using 3 x 1 ml of hexane and analysed by HPLC.

15) A kit for testing for the presence of organophosphate pesticides using gMAB 1507

A kit for testing for the presence of organophosphates, particularly chlorpyrifos, chlorfenvinphos, tetrachlorfenvinphos, ethyl paraoxon, demeton-S-methyl and CPT was provided as follows:

iMAB 1901 was conjugated to HRP by the periodate method. HRP (10mg) dissolved in distilled water (2.5 ml) was reacted with 0.1 M sodium periodate (0.5 ml) stirring for 1.5 hrs at room temperature. The mixture was dialysed against 1 mM sodium acetate pH 4.4 over 18 hrs. Purified MAB (2 mg) was dissolved in 0.25 M carbonate buffer (2ml) pH 9.4. added to the dialysate and stirred for 4h at room temperature in the dark. Sodium borohydride (0.2 ml of a 4 mg/ml solution) was added and allowed to stand at 4C overnight. The labelled antibody was dialysed against 3 X 11 of PBS, concentrated to 1 ml on an Amicon filter, sterilized through a 22 syringe filter, and the volume made up to 10 ml with a

preservative solution containing bovine serum albumin (BSA) and thiomersal, before being stored at 4°C.

In use, immobilised gMAB 1507 is exposed to HRP-labelled iMAB 1901 in the presence of a sample suspected of containing organophosphate pesticides. The presence of the pesticides is deduced by measurement of the amount HRP-labelled iMAB 1901 being displaced from gMAB 1507, the HRP activity being tested as in 6).

An alternative kit was provided using the pesticide-coated plates described in 12), gMAB 1507 and the anti-mouse IgG-alkaline phosphatase conjugate of 7). Binding of gMAB 1507 to the plate in the presence and absence of the sample was assessed using the conjugate as described in 7), binding being reduced in the presence of pesticide.

EXAMPLE 2 - AN INTERMEDIATE MAB HAVING SPECIFIC BINDING AFFINITY FOR VETERINARY DRUGS (ANTIBIOTICS)

The immunisation protocol and preparation of iMABs was as in the 1st example. Cell lines were screened as follows: from an initial 120 seeded fusion plate wells, 6 were selected which were shown to contain hybridomas secreting antibody that both bound to a ß-lactamase coated plate and were displaced from this plate by 1mg/ml penicillin G. Subsequent cloning of these hybridomas produced 6 stable cell lines. The antibodies from these cell lines were checked to ensure that they were true inhibitors of ß-lactamase as follows: the effect of 100 µl supernatant was assessed on an activity assay based on 1µg nitrocefin and 22 mU penicillinase in a final volume of 300 µl. Results are showed between 14 and 57% inhibition, strongly suggesting that one or more of the antibodies interact with the active cite of the enzyme. Such antibodies may thus be used, in methods analogous to those in Example 1, as immunogens to produce generic antibodies for use in drug screening.

CLAIMS

1. A method for producing generic antibodies having a specific binding affinity for two or more of a class of compounds demonstrating a common mode of biological activity comprising:

(a) selecting a receptor or enzyme through which the class of compounds exert the biological activity,

(b) raising monoclonal antibodies against the recognition site of the receptor or enzyme,

(c) inoculating an animal with these monoclonal antibodies or fragments thereof, and

(d) recovering the generic antibodies from a body fluid or tissue of the animal.

2. A method as claimed in claim 1 wherein the two or more compounds are bound with relative affinities in the range of 1 to 10^4.

3. A method as claimed in claim 1 or claim 2 wherein the generic antibodies have specific binding affinity for at least four compounds.

4. A method as claimed in any one of claims 1 to 3 wherein the compounds are inhibitors of the receptor or enzyme.

5. A method for producing cells capable of producing generic monoclonal antibodies having a specific binding affinity for two or more of a class of compounds demonstrating a common mode of biological activity comprising:

(a) selecting a receptor or enzyme through which the class of compounds exert the biological activity,

(b) raising monoclonal antibodies against the recognition site of the receptor or enzyme,

(c) inoculating an animal with these monoclonal antibodies or fragments thereof,

(d) recovering cells capable of producing generic antibodies from the animal, and

(e) immortalising the cells.

6. A method for producing generic monoclonal antibodies having a specific binding affinity for two or more of a class compounds demonstrating a common mode of activity comprising culturing the cells produced by the method of claim 5 and isolating generic monoclonal antibodies from the culture medium.

7. Generic polysera obtainable by the method of any one of claims 1 to 4.

8. Cells obtainable by the method of claim 5.

9. Generic antibodies in isolated form obtainable by the method of any one of claims 1 to 4 or claim 6.

10. Modified cells or generic antibodies obtainable from the cells or generic antibodies of claim 8 or claim 9.

11. A method of screening a sample for the presence of absence of compounds demonstrating a common mode of activity comprising:

(a) contacting the sample with generic antibodies as claimed in claim 9 under conditions sufficient to permit the formation of immune complexes,

(b) detecting the formation of the immune complexes, and

(c) relating the presence or absence of immune complexes to the presence or absence of the compounds.

12. A method as claimed in claim 11 wherein the immune complexes are detected by displacement of a labelled marker from the generic antibodies.

13. A method for extracting compounds demonstrating a common mode of activity from a sample comprising:

(a) contacting the sample with generic antibodies as claimed in claim 9 under conditions sufficient to permit the formation of immune complexes, and

(b) treating any immune complexes formed such as to as to dissociate the compounds therefrom.

14. A kit for use in the methods of claims 11 to 13 comprising one or more of: the generic polysera of claim 7; the generic antibodies of claim 9 or 10; means for detecting the formation of immune complexes; means for dissociating compounds from immune complexes.

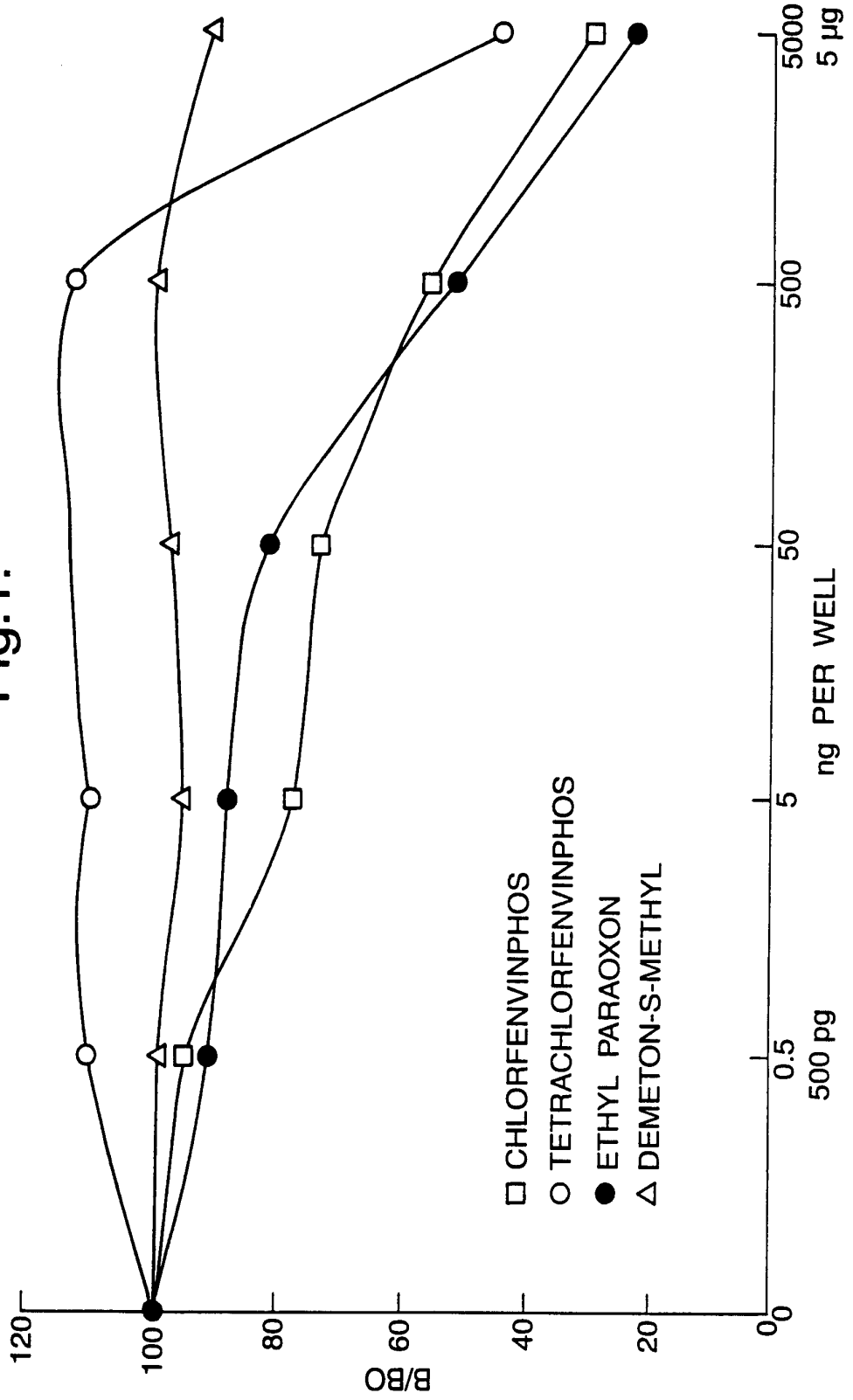
15. A method, generic polysera, cells, generic antibodies or a kit as claimed in any one of claims 1 to 14 wherein the compounds are selected from the group consisting of: organophosphates; antibiotics.

16. A method, generic polysera, cells, generic antibodies or a kit as claimed in claim 15 wherein the compounds are organophosphates which are pesticides.

17. A method, generic polysera, cells, generic antibodies or a kit as claimed in claim 15 or claim 16 wherein the monoclonal antibodies used to raise the generic antibodies are raised against an enzyme or receptor which binds or is inhibited by organophosphates.

18. A method, generic polysera, cells, generic antibodies or a kit as claimed in any one claims 15 to 17 wherein the monoclonal antibodies used to raise the generic antibodies are raised against cutinase.

Fig.1.


B/BO
120
100
80
60
40
20
0
0.5
500 pg
5
50
500
5000
5 µg
ng PER WELL
□ CHLORFENVINPHOS
○ TETRACHLORFENVINPHOS
● ETHYL PARAOXON
△ DEMETON-S-METHYL