

Efficient purification of unique antibodies using peptide affinity-matrix columns

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Abstract

Phage display technology was used to identify peptide ligands with unique specificity for a monoclonal model antibody, MK16, that recognises the human multiple sclerosis associated MHC class II molecule DR2 in complex with a myelin basic protein (MBP)-derived peptide corresponding to residue 85–99. Several peptide epitopes were identified and all of them recognised specifically MK16. One peptide, ER6.1, was selected and linked to beaded agarose and demonstrated excellent performance as a peptide affinity chromatography matrix. This epitope matrix was efficient in the purification of MK16 Fab fragments and had no affinity for other antibodies. Using this peptide matrix MK16 IgG could be purified from cell culture supernatants thereby separating MK16 IgG from bovine IgG normally present in the enriched growth media used for such cells.

Investigations of the fine specificity of the ER6.1 peptide demonstrated that it recognised a unique epitope within the heavy chain CDR3 region of the MK16 antibody. Thus, variants of MK16 antibody, which had retained the specificity and affinity of the original antibody but had slightly different amino acid composition in the CDR3 region, were not recognised by the ER6.1 peptide. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Established protocols for the purification of monoclonal antibodies take advantage of affinity ligands directed against general epitopes on antibodies (e.g., protein A and protein G), but these techniques cannot distinguish between antibodies with different specificities.

This can be a problem when purifying Fab fragments, scFv or full-size antibodies from cell culture supernatants. Thus, serum antibodies are often present in cell culture media due to the presence of fetal calf serum (FCS) used as a supplement and such antibodies will be co-purified with the monoclonal antibody produced by the cells.

Peptides have been used previously as ligands in affinity purification of antibodies. Thus, peptides directed against general epitopes not differing between antibodies of different specificities have been selected

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and used as an affinity matrix (Ehrlich and Bailon, 2001; Jacobs et al., 2003). Others have used peptide ligands specific for a unique antibody based on an already known epitope (Murray et al., 1997). New peptide epitopes specific for a monoclonal antibody have been identified using biological or artificial peptide libraries in combination with different selection techniques (Murray et al., 2001; Smith et al., 2002). However, in some of these cases refinement of the peptide was required to make it useful as an affinity matrix.

The aim of the present study was to explore the possibility of using peptides for the affinity purification of selected antibodies from cell culture supernatant containing irrelevant serum antibodies. As a model system we identified relevant peptides by biopanning phage peptide libraries against MK16 Fab fragments isolated by conventional procedures. One of these peptides, ER6.1, was used for the purification of MK16 in the Fab or IgG format from cell culture supernatants.

MK16 is a murine antibody directed against the multiple sclerosis associated human MHC class II molecule HLA-DR2b (DR2b) in complex with a myelin basic protein (MBP)-derived peptide corresponding to residues 85–99 (MBP 85–99) (Krogsgaard et al., 2000). DR2b is thought to play a key role in the pathogenesis of multiple sclerosis (MS) by presenting auto-antigens such as MBP-derived peptides to auto-aggressive T cells (Madsen et al., 1999; Steinman, 1996). This is further corroborated by the finding that DR2b-MBP 85–99 complexes have been visualised in brain lesions of MS patients using the MK16 antibody (Krogsgaard et al., 2000).

Our results demonstrate a general method to identify peptides unique for monoclonal antibodies and the use of such peptides for the purification of unique antibodies from mixtures of antibodies.

2. Materials and methods

2.1. Expression and purification of Fab molecules

The Fd (heavy chain of the Fab fragment) and light chain encoding regions of the various Fab fragments were cloned in a standard T7 polymerase-driven expression vector (pET, Novagen), transformed into *E.*

coli BL21 host cells, and inclusion bodies were produced according to published procedures (Garboczi et al., 1992). The purified inclusion body material of Fd and light chain was reduced by adding 1,4-Dithioerythritol (DTE) to a final concentration of 65 mM and then mixed and refolded in a redox buffer (0.1 M Tris–HCl, 0.5 M L-arginine–HCl, 0.9 mM GSSG, 2 mM EDTA; pH = 7.5–8.5) at 10 °C for 48–72 h. Following a filtration step through a 0.45-µm Nitrocellulose filter (Millipore), the refolding mixture was concentrated using an Amicon 8400 concentrator (Millipore) and a Centricon Plus20 spin column (Millipore). The Fab fragments were subsequently purified by size separation on a Sephadex column (Superdex™ 200, Amersham Biosciences) using Äkta FPLC (Amersham Biosciences) equipment with a flow rate of 0.5 ml/min and PBS as eluent.

2.2. Biopanning

A phage library displaying seven amino acids in random sequence order at the N-terminal end of pIII (New England Biolab) was used for affinity selection of peptide binders to MK16 Fab. Both a linear and a constrained version of the peptide library were used for the selection. Microtiter plates (Maxisorb, NUNC) were coated at 4 °C for 12–16 h with purified MK16 Fab at 40 µg/ml using 100 µl per well. These plates were subsequently washed in PBS, pH = 7.4 supplemented with 0.05% Tween-20 and then blocked with 2% skimmed milk/PBS, pH = 7.4 for 2 h at room temperature. Bacteriophages at 10¹¹ pfu/100 µl were used for each panning round. The constrained and linear libraries were mixed and panned together as a mixture in 2% skimmed milk/PBS, pH = 7.4. After 1-h incubations at room temperature bound phages were eluted with glycine/HCl, pH = 2.2 for 10 min followed by neutralisation with Tris–HCl, pH = 9.0. After three to six rounds of panning, single clones were isolated, DNA extracted and sequenced in the region corresponding to the random peptide region using an ABI-prism DNA sequencer.

2.3. ELISA

2.3.1. Phage ELISA

100-µl portions of LBJ58, MK16, PS35 and PS50 Fab fragments or full-size MK16 IgG were adsorbed

onto the wells of a microtiter plate (96 well NUNC) at 1 µg/ml in PBS, pH=7.4 at 4 °C for 12–16 h. The following washes and incubations were performed at room temperature. After blocking in 2% skimmed milk/PBS, pH=7.4 for 2 h the plates were washed three times in washing buffer (PBS +0.05% tween-20; pH=7.4). 100 µl of a solution of the ER6.1 peptide phage was added in a concentration of 1×10^{10} pfu/ml and further incubated for 2 h. Following five washings, 100 µl of peroxidase conjugated mouse anti-M13 monoclonal antibody (Amersham Pharmacia Biotech) diluted 1/5000 in 2% skimmed milk/PBS, pH=7.4 was added and incubated for 1 h. The plates were washed five times and detection was carried out by adding 100 µl of a chromogene solution (1 OPD-tablet (DAKO) + 5 µl 30% H₂O₂ per 3 ml of water). The reactions were terminated 15–25 min later by adding 150 µl of 1 M H₂SO₄. Absorbance values (*A*) were measured at 490 nm using an immunoreader (Emax).

2.3.2. Detection of MK16 IgG during peptide affinity purification from cell culture supernatant

The wells of a microtiter plate were coated with 100 µl of a 1 µg/ml solution of either the DR2b-MBP 85–99 (MBP 85–99 is a peptide derived from myelin basic protein corresponding to residue 85–99) or DR2b in complex with an irrelevant peptide, DQw6 43–58 (peptide containing residue 43–58 of HLA-DQw6) in PBS, overnight at 4 °C and then blocked for 2 h in 2% skimmed milk/PBS at room temperature. Both complexes were generated as described in Section 2.7. The protein concentration in the eluate fractions and flow-through from the ER6.1 peptide purification of MK16 IgG from cell culture supernatant were adjusted to 30 µg/ml by the addition of PBS. 100 µl of these samples were added to each well and incubated for 2 h at room temperature. Bound antibodies were detected by the addition of 100 µl of peroxidase conjugated goat-anti-mouse Ig (Fab specific, from Sigma) diluted 1/50,000 in 2% skimmed milk/PBS. Quantitation and washes between incubations were carried out as described for phage ELISA.

2.3.3. Competition assay

The wells of a microtiter plate were coated with 100 µl of a 1 µg/ml solution of the DR2b-MBP 85–99

complex in PBS. Various concentrations of chemically synthesised ER6.1 peptide (Schäfer-N, Copenhagen) were incubated with 3 µg/ml of different Fab fragments for 30 min in round-bottomed microtiter plates before transfer to the DR2b-MBP 85–99 coated microtiter wells and further incubation for 2 h at room temperature. Bound Fab fragments were detected by incubation with biotin conjugated anti-mouse Ig (DAKO) diluted 1/5000 in 2% skimmed milk/PBS pH=7.4 for 1 h followed by a fourth layer of Europium conjugated streptavidin (Wallac) diluted in assay buffer (Wallac) for 45 min. Quantitation was carried out after the addition of Enhancement Solution (Wallac) and the released Eu³⁺ was measured using a time resolved fluorometer. All incubation volumes were 100 µl and the reactions were carried out at room temperature. Between the addition of each layer the microtiter plate was washed six times in 0.05% Tween-20/PBS, pH=7.4.

2.4. Production of MK16IgG

The murine antibody MK16 was originally prepared by phage display technology (Krogsgaard et al., 2000) in the Fab format. The variable heavy and light chain encoding regions (Fv) of MK16 Fab were recloned in two separate eukaryotic expression vectors pLNOH2 and pLNOK Zeo (Norderhaug et al., 1997) together with chromosomal fragments encoding constant regions of heavy and light chain that was PCR-amplified from cells isolated from a normal mouse liver. These two expression vectors were co-transfected into CHO cells using Lipofectamine Plus (Invitrogen) according to the supplied protocol. The transfected CHO-cells were grown in HAMS F-12 media (Gibco) supplemented with 10% fetal calf serum (FCS) and 800 µg/ml geneticin (Invitrogen). One clone producing high amounts of MK16 IgG was selected and expanded. Full-size MK16IgG was purified from the cell culture supernatant.

2.5. Chromatographic methods

2.5.1. Protein A column chromatography

Typically, 500-ml cell culture supernatants from MK16 IgG expressing CHO-cells were concentrated to approximately 40 ml using an Amicon Concentrator from Millipore and 40 ml Binding buffer was

added (1.5 M Glycine/NaOH, 3 M NaCl pH=9.0). This sample was filtered by passage through a 0.22- μ m cellulose acetate filter (Millipore) and 20-ml portions were loaded into the Prosep-A spin column (Millipore) previously equilibrated with 10 ml Binding buffer. The spin column was centrifuged at $150 \times g$ for 20 min in a Sigma centrifuge. Loading and centrifugation were repeated until all the concentrated supernatant was processed. The column was washed three times with 10 ml Binding buffer by centrifugation for 2 min at $500 \times g$. Bound antibody was eluted in two steps by 10 ml 0.1 M sodium citrate, pH=5.5 and 10 ml 0.2 M Glycine/HCl, pH=2.5, respectively. Eluted material was neutralized by adding 1 M Tris-HCl, pH=9.0.

2.5.2. Peptide affinity purification of MK16

The peptide ER6.1 recognising the antigen-binding domain of MK16 was synthesised using Fmoc chemistry and covalently linked to activated CH-Sepharose (Amersham Biosciences) according to the general protocol provided by the supplier. The ER6.1 peptide was coupled to the Sepharose beads via an extra glycine inserted at its N-terminal end, thereby forming an amide-bond between the glycine amino group and the carboxyl group on the activated Sepharose beads. Alternatively, the ER6.1 peptide was synthesised with an N-terminal hexanoic acid extrusion and the extended peptide was subsequently linked to the CH-Sepharose beads via the N-terminus as described before. The peptide was synthesised and coupled to Sepharose beads by Schäfer-N, Copenhagen. 0.5 ml of the peptide coupled Sepharose beads was packed into a column (Poly-Prep Column, Bio-Rad). Eluted antibody from the protein A chromatography was loaded onto the peptide affinity column by gravity and the column was subsequently washed three times with 1 ml PBS, pH=7.4. Bound MK16 was eluted with 2×1 ml 0.2 M Glycine/HCl, pH=2.5. Eluted material was neutralized by adding 1 M Tris-HCl, pH=9.0.

2.6. SDS-PAGE

Electrophoresis was performed in a Bio-Rad Mini protean II system using Ready Gels, 12% Tris-HCl from Bio-Rad. 15- μ l samples were added to either 5- μ l nonreducing (62.5 mM Tris-HCl pH=6.8, 2%

SDS, 0.006% Bromophenol blue, 10% glycerol) or reducing (non-reducing buffer +0.05% β -mercaptoethanol) loading buffer and boiled for 5 min at 96 °C before loading onto gels.

Gels were stained with GelCode®Blue Stain Reagent (Bio-Rad).

2.7. Generation of DR2b-peptide molecules

DR2b molecules were produced and loaded with peptide as previously described (Hansen et al., 1998). Briefly, S2 cells co-transfected with the DR2b α -chain (DRA1*0101) and β -chain (DRB1*1501) were grown in Drosophila-SFM medium (Life Technologies) supplemented with Penicillin/Streptomycin 100 units/ml (Life Technologies) and Glutamax-I supplement (Life Technologies) at 25 °C. After CuSO₄ induction detergent-solubilised HLA class II molecules were purified by affinity chromatography using a column consisting of an anti-human MHC class II antibody (L243) immobilised on Sepharose beads. The purified protein was characterised by SDS-PAGE and the protein concentration determined in a BCA protein assay (Pierce) using BSA as reference protein.

DR2b molecules were loaded with various peptides by incubating the affinity-purified DR2b with 75–100 molar excess of peptide at 25 °C for 48 h in the presence of protease inhibitor (Complete, Boehringer Mannheim) and 1% OG (*n*-Octyl β -D-Glucopyranoside) (Sigma) in PBS, pH=7.4. Following incubation, nonbound peptides were removed by gel filtration on a G25-Sephadex spin column containing PBS, pH=7.4 and 0.1% Igepal (Sigma).

3. Results

3.1. Biopanning against MK16 Fab

MK16 Fab molecules were generated as described in Materials and methods and immobilised to the wells of a microtiter plate. Biopanning was performed using two phage peptide libraries displaying seven amino acids in random sequence at the N-terminal end of pIII, a linear and a cysteine-constrained version (New England Biolab). Both libraries contain a short linker sequence (Gly-Gly-Gly-Ser) between the displayed peptide and pIII and encode 2×10^9 indepen-

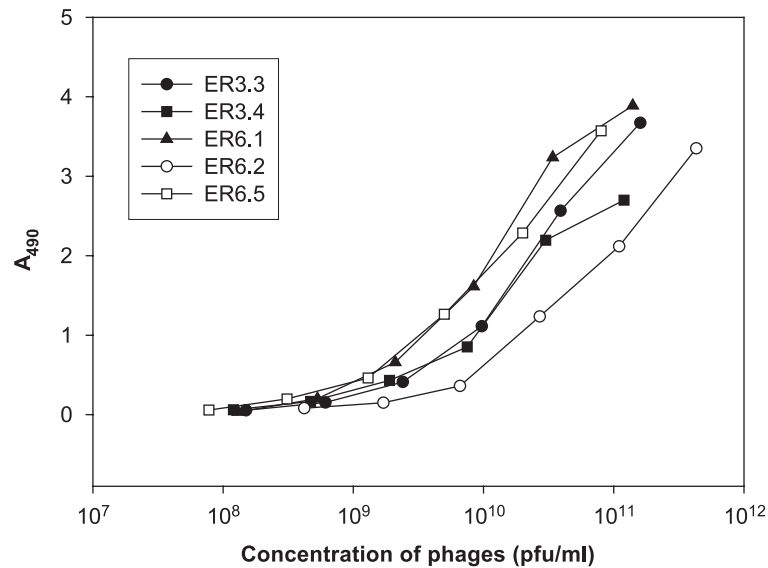


Fig. 1. ELISA of MK16 specific binders. A microtiter plate coated with MK16 Fab was incubated with peptide phages in various concentrations representing each of the conserved motifs obtained from the biopanning. Bound phages were detected with peroxidase labelled anti-M13 antibody. Each symbol represents a single determination.

dent peptide sequences. Six rounds of panning were performed and a 100-fold enrichment of phages recognizing MK16 Fab was observed already after three rounds of panning. Individual clones from panning rounds three and six were randomly picked and analysed by ELISA for binding to MK16 Fab. The ELISA results from the five most positive clones are shown in Fig. 1. DNA from each clone was isolated and sequenced and as shown in Table 1 all binders derived from the constrained library. No unique consensus sequence was observed but several regions of identity were evident in different pairs of clones.

Table 1

Name and amino acid sequence of peptides displayed on phages and selected for binding to MK16 Fab. Regions of identity in pair-wise comparisons are underlined

Name	Peptide sequence
ER3.3	<u>CSISTHSWC</u>
ER6.3	<u>CSIKTHSWC</u>
ER6.5	<u>CNYKTHSWC</u>
ER3.4	CYGNWFNSNC
ER6.1	<u>CNYSVAHLC</u>
ER6.2	<u>CKWSVAHLC</u>

One peptide phage was selected (ER6.1) based on its reactivity in ELISA and expected stability when handled as a free peptide. This peptide-phage was analysed in ELISA for reactivity with different antibody fragments (Fig. 2). ER6.1 demonstrated high reactivity with MK16 Fab but did not bind to a pool of different full-size mouse IgG antibodies

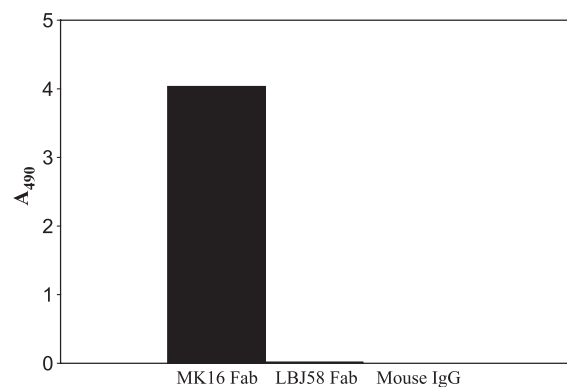


Fig. 2. Specificity of ER6.1 peptide phages analysed by ELISA. A microtiter plate was coated with MK16Fab, LBJ58 Fab or a pool of mouse IgG. The reactivity of ER6.1 peptide phages with the various antibodies was determined in an ELISA setup using 5×10^9 pfu per well.

(DAKO, Denmark). Additionally, ER6.1 could distinguish between two murine Fab fragments directed against very similar targets, since no reactivity was observed with LBJ58 Fab, which is directed against the same MHC as MK16 (DR2b) but in complex with the peptide PLP 95–116 derived from Proteolipid Protein (results to be published elsewhere). These data demonstrate that the ER6.1-phage specifically recognises MK16 Fab and not other murine antibodies or Fab fragments even though they have highly comparable specificities.

3.2. Application of the ER6.1 peptide as an affinity ligand

Synthetic ER6.1 peptide was coupled to Sepharose beads to produce an immunoaffinity matrix. The matrix was packed into a column and MK16 Fab, human IgG or LBJ58 Fab loaded onto the column. The column was washed extensively before elution of bound protein. The protein concentration was determined in the flow-through, the wash and the eluate to evaluate the binding capacity of the antibody fragment to the peptide column (Table 2).

Human IgG and LBJ58 Fab had no affinity for the column since all protein material could be found in the flow-through and washing steps. However, 54% of MK16 Fab was recovered in the eluate while 46% was detected in the wash and flow-through. The loading efficiency of MK16 Fab could be improved by increasing the volume of the column (results not shown). The binding capacity was estimated to be about 1 mg of specific Fab material per milliliter of peptide matrix material.

In an attempt to improve the accessibility of the ER6.1 peptide on the surface of the Sepharose beads for recognition by the MK16 antibody the peptide was

synthesised with a hexanoic acid extension, which was used to couple the peptide to the bead material. This “elevation” of the ER6.1 peptide resulted in a fivefold improvement in the binding capacity of the MK16 antibody to the peptide-matrix material without compromising the binding specificity (results not shown).

3.3. Affinity chromatography of antibodies from cell culture supernatant

We next investigated whether the matrix could be used to isolate MK16 IgG produced from transfected CHO-cells. The cells produced MK16 IgG as a soluble molecule to be found in the supernatant. The CHO-cells were grown in serum-rich media containing many proteins including antibodies of unknown specificities.

Initially, MK16IgG from cell culture supernatant was pre-purified on a protein A column. As a control, fresh culture medium (supplemented with FCS and relevant antibiotics) was purified using the same procedure. The protein A column step was introduced to get rid of most of the nonantibody proteins in the cell culture medium and to evaluate the serum antibodies present in the medium. The purified samples were analysed by SDS-PAGE under reducing conditions (Fig. 3). Protein bands corresponding to heavy and light chain of antibody fragments were found in both samples. As expected the serum antibodies from

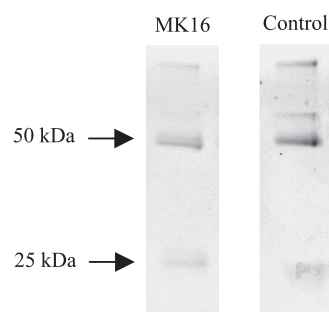


Fig. 3. Protein A purification of MK16 IgG from cell culture supernatant. MK16IgG in cell culture supernatant was protein A purified and the eluted fraction analysed by SDS-PAGE under reducing conditions. Culture medium that had not been in contact with antibody producing cells was used as a control and purified and analysed using the same procedure. Approximate size of the bands corresponding to antibody heavy and light chains is indicated by the two arrows.

Table 2

One milliliter of 1 mg/ml solutions of different antibodies was loaded onto a 0.5-ml ER6.1-peptide column. The protein concentration was determined by spectrophotometry. The amount of protein is shown as percent protein detected relative to the total amount of protein loaded

	MK16 Fab	LBJ58 Fab	Human IgG
Flow-through	28%	79%	70%
Wash	18%	21%	30%
Eluate	54%	0%	0%

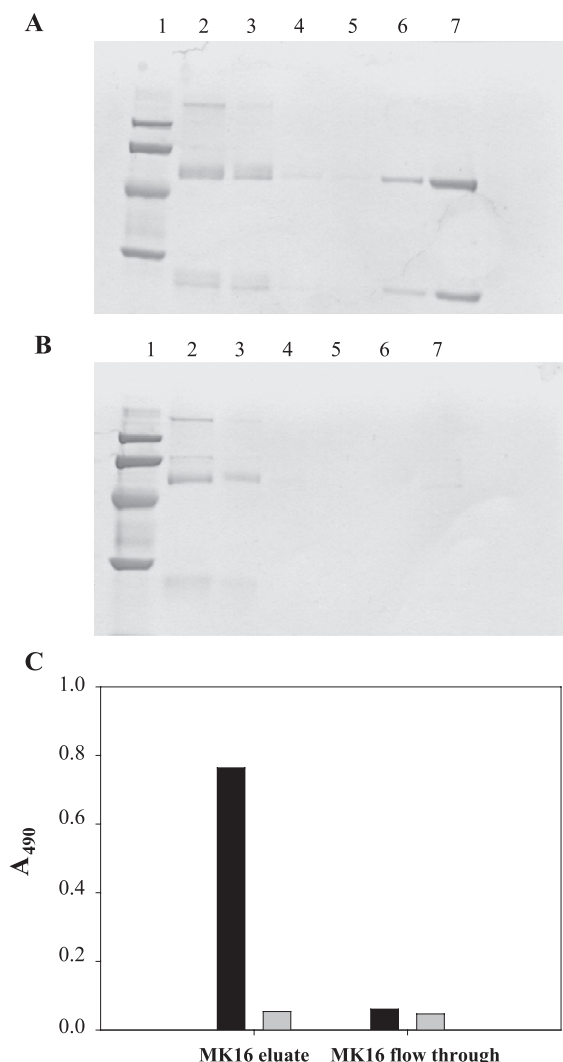


Fig. 4. Analysis of ER6.1 peptide purification. SDS-PAGE analysis of MK16 IgG from cell culture supernatant (A) and serum antibodies (B) purified using columns of the ER6.1 peptide coupled to Sepharose beads. Lane 1: low molecular weight standard (Biorad), lane 2: flow-through, lanes 3–5: wash, lanes 6–7: eluted antibodies. Both samples had previously been protein A purified. (C) ELISA analysis of peptide purification of the MK16 sample shown in (A). 100- μ l samples of eluate or flow-through in a concentration of 30 μ g/ml protein were analysed for reactivity with DR2b-MBP85-99 (black bars) or DR2b-DQw6 43-58 (grey bars). Bound antibodies were detected with peroxidase labelled anti-Ig antibody as described in Materials and methods. Three ELISA experiments were performed, here a representative set of data are shown where each bar illustrates a single determination.

Table 3

DNA sequence (red) and amino acid sequence (black) of CDR3 heavy chain region of the Fab fragments MK16, PS35 and PS50

MK16	TGG	ACT	ACG	GCT	TCT	GAC	TAC
	W	T	T	A	S	D	Y
PS35	CAG	ACG	ACG	TCT	AAG	GAC	AAT
	Q	T	T	S	K	D	N
PS50	GAG	ACT	ACG	TCT	CAG	GAC	TAC
	E	T	T	S	Q	D	Y

the culture medium and MK16 IgG bound to the protein A column.

The protein A purified products were then applied onto the ER6.1 peptide matrix. After extensive washings bound antibodies were eluted with a low pH buffer. Samples from each purification step were analysed by SDS-PAGE (Fig. 4A+B). Antibodies were eluted from the ER6.1 matrix when the MK16 IgG sample was applied but no material could be detected in the eluate of the culture medium sample. In both samples antibody heavy and light chain could be detected in the flow-through. An ELISA test demonstrated that the antibodies detected in the eluate of the MK16 IgG sample reacted with DR2b-MBP 85–99, while no reactivity to DR2b-MBP 85–99 was seen with the flow-through fraction of the control sample (Fig. 4C). This confirms that the eluted antibodies must be MK16IgG and the antibodies detected in the flow-through originated from the supplemented FCS. These data clearly demonstrate that the ER6.1

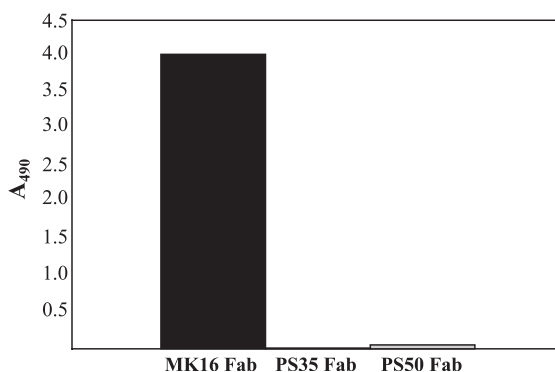


Fig. 5. Reactivity of ER6.1 peptide phage with MK16 derivatives. Fine specificity of ER6.1 peptide phage was analysed in ELISA. Wells in microtiter plates were coated with either MK16, PS35 or PS50 Fab. The reactivity of ER6.1 peptide phage with the various antibodies was determined using 100- μ l samples of a phage solution of 1×10^{10} pfu/ml.

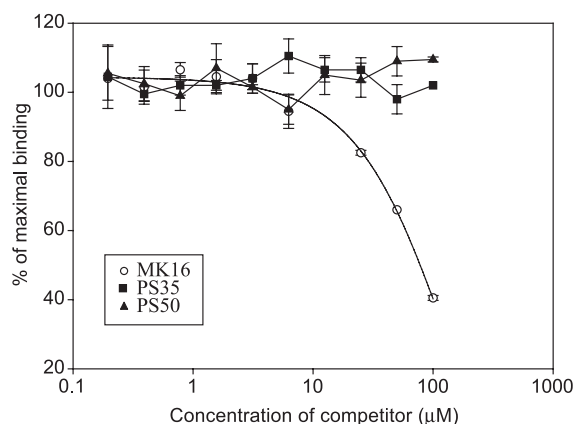


Fig. 6. ER6.1 recognises the CDR3 region of MK16. Microtiter wells coated with DR2b-MBP 85–99 complexes were incubated with a fixed concentration of MK16, PS35 or PS50 Fab and various concentrations of synthetic ER6.1 peptide. Bound antibody was detected with biotin conjugated anti-mouse antibody (DAKO) and visualised using Europium conjugated Streptavidin (Wallac). For each concentration the mean and the standard deviation of the measurements are shown ($n=2$).

matrix selectively isolates MK16IgG from other antibodies present in the culture media.

3.4. Fine specificity of ER6.1 peptide

To investigate which part of MK16 Fab is recognised by the ER6.1 peptide an ELISA experiment with two other antibodies (PS35 Fab and PS50 Fab) was performed. PS35 and PS50 recognise DR2b-MBP 85–99 in a peptide-specific and DR2b-restricted manner similar to that of MK16 and with similar affinity (to be published elsewhere). PS35, PS50 and MK16 have identical light chains and almost identical heavy chains only differing in the amino acid sequence in the CDR3-region as shown in Table 3.

No reactivity could be detected with either PS35 or PS50 Fab (Fig. 5). The major antigen recognising part of an antibody is believed to be the heavy chain CDR3 region. The ELISA results with PS35 and PS50 indicate that the ER6.1 peptide is directed against the antigen binding part of MK16 Fab, since the binding is affected when only a few amino acids are changed in the CDR3 region of the heavy chain. To further investigate this assumption the synthetic ER6.1 peptide was used in a competition assay. As shown in Fig. 6 the binding of MK16 to immobilised

DR2b-MBP85–99 was inhibited with the ER6.1-peptide in a specific and dose-dependent way. However, the ER6.1 peptide did not block the binding of PS35 and PS50 to DR2b-MBP85–99. This confirms that the ER6.1 peptide binds to the antigen-recognising part of MK16 Fab and this binding is highly specific for MK16. It should be emphasized that the results depicted in Fig. 6 only illustrate the effect of the free and soluble ER6.1 peptide on the reaction between the MHC-peptide target and different antibodies recognising this target. The affinity between soluble ER6.1 and MK16 (about 90 μ M as judged from the apparent K_D value) cannot be related to the loading capacity of the ER6.1 column for binding of MK16 as this reflects avidity effects generated by the coating density of the ER6.1 peptide coupled to the beads.

This very selective recognition of MK16 Fab by the ER6.1 peptide could be a unique feature of this particular peptide. However, several peptide phages with specificity for MK16 were isolated by our selection strategy. Analysis in ELISA of these clones revealed that they had affinity for the heavy chain CDR3 region of MK16 since all clones demonstrated no or only weak reactivity with PS35 and PS50 (Fig. 7). Therefore our selection method appears to identify peptide phages specifically directed against unique parts of the monoclonal antibodies making the method very useful in the purification of selected monoclonal antibodies.

4. Discussion

Many monoclonal antibodies are produced in mammalian cell systems grown in media supplemented with FCS and consequently contain bovine serum antibodies. Conventional methods for antibody purification such as protein A or G do not distinguish between the antibodies produced by the antibody expressing cells and the bovine serum antibodies (Jacobs et al., 2003). Proteins A and G bind murine, bovine and human IgGs, making it difficult to separate these antibodies from each other (Eliasson et al., 1989). The work presented here demonstrates a method to overcome this problem. We have used phage display to isolate peptide mimotopes specific for the model monoclonal antibody MK16. We used a unique set of antibodies

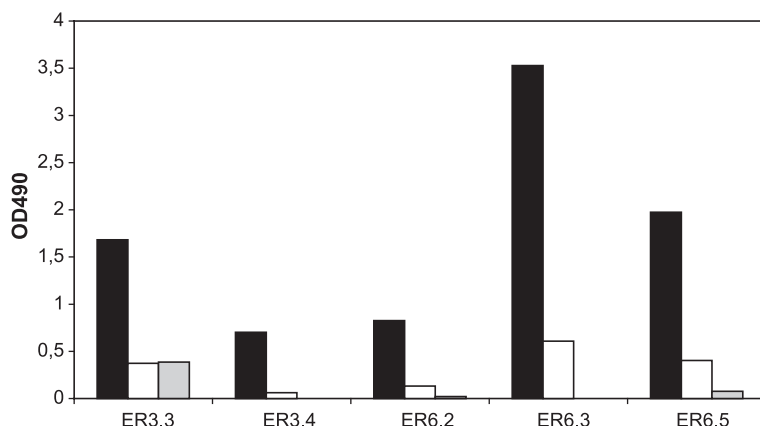


Fig. 7. All isolated peptide binders against MK16 are directed against the CDR3 region. MK16 positive peptide phages were analysed in ELISA for reactivity with MK16 Fab (black bars), PS50 Fab (grey bars) or PS35 Fab (open bars). A concentration of 5×10^{10} phages/ml was used. Each bar corresponds to a single determination in a representative experiment.

(MK16, PS35 and PS50) to demonstrate that the peptides we have identified are directed to a part of MK16 that is absolutely specific for this antibody and not others. We showed that one of the peptides, ER6.1, could be used for affinity chromatography purification of MK16 in the Fab format as well as a complete IgG produced by mammalian cells. The ER6.1 peptide affinity matrix was capable of separating our model antibody MK16 IgG from the bovine antibodies also present in the cell culture media.

Several other peptides with unique specificity for MK16 identified in this work seem to have affinities comparable to that of ER6.1 and could therefore also be used as affinity ligands. Combining the different peptides in a mixed affinity matrix is feasible and would most likely increase the loading capacity of the peptide matrix.

The peptide library screening and our selection protocol seem to be an efficient and relatively fast method for the isolation of peptide mimotopes unique for a single monoclonal antibody. It is likely that this method would be applicable to other antibodies directed against simple as well as complex targets.

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