Cancer Immunol Immunother (2001) 50: 93–101 © Springer-Verlag 2001

ORIGINAL ARTICLE

**Cindy Wong · Robert Waibel · Michael Sheets
Jean-Pierre Mach · Ricarda Finnern**

Human scFv antibody fragments specific for the epithelial tumour marker MUC-1, selected by phage display on living cells

Received: 2 November 2000 / Accepted: 11 January 2001

Abstract New anti-cancer agents are being developed that specifically recognise tumour cells. Recognition is dependent upon the enhanced expression of antigenic determinants on the surface of tumour cells. The tumour exposure and the extracellular accessibility of the mucin MUC-1 make this marker a suitable target for tumour diagnosis and therapy. We isolated and characterised six human scFv antibody fragments that bound to the MUC-1 core protein, by selecting a large naive human phage display library directly on a MUC-1-expressing breast carcinoma cell line. Their binding characteristics have been studied by ELISA, FACS and indirect immunofluorescence. The human scFv antibody fragments were specific for the tandem repeat region of MUC-1 and their binding is inhibited by soluble antigen. Four human scFv antibody fragments (M2, M3, M8, M12) recognised the hydrophilic PDTRP region of the MUC-1 core protein, which is thought to be an immunodominant region. The human scFv antibody fragments were stable in human serum at 37 °C and retained their binding specificity.

For imaging or targeting to tumours over-expressing MUC-1, it might be feasible to use these human scFv, or multivalent derivatives, as vehicles to deliver anti-cancer agents.

Key words Human scFv · Mucin MUC-1 · Phage display · Cell surface selection · Tumour immunotherapy

Abbreviations *CDR* Complementarity determining region · *FR* framework · *mAb* monoclonal antibody · *scFv* single-chain Fv antibody fragment · *VNTR* variable number of tandem repeats

C. Wong · J.-P. Mach · R. Finnern
University of Lausanne, Department of Biochemistry,
1066 Epalinges, Switzerland

R. Waibel
Paul Scherrer Institute,
5232 Villigen PSI, Switzerland

M. Sheets
University of San Francisco,
San Francisco General Hospital,
Department of Anesthesia,
San Francisco, CA 94110, USA

R. Finnern (✉)
RWTH-Aachen,
Biology I, Worringerweg 1,
52074 Aachen, Germany
e-mail: finnern@molbiotech.rwth-aachen.de
Tel.: +49-241-8099399; Fax: +49-241-871062

Introduction

More than 90% of breast cancers show an increased and under-glycosylated expression of the membrane-bound mucin molecule MUC-1 [3]. The complete amino acid sequence of the core protein of the *MUC-1* gene product has been obtained by gene cloning and shows the mucin to be a transmembrane glycoprotein composed primarily of a large extracellular domain made up of a VNTR of 20 amino acids in length (PDTRPAPGSTAPPAH-GVTSA), a transmembrane domain and a cytoplasmic tail [11, 29]. On normal epithelial cells, it is characterised by polarised apical surface expression and extensive glycosylation with mostly O-linked carbohydrates. Non-polarised expression and aberrant glycosylation of this molecule on tumour cells leads to the exposure of novel tumour-specific peptide epitopes, located in the VNTR, recognised by both mouse and human antibodies [12] and cytotoxic T cells [16]. Depending on the number of tandem repeats present in the mucin of different individuals, this epitope can be repeated 2–100 or more times per molecule. Moreover, the three-dimensional structure of the repeat is very rigid, with each epitope being not only a sequence repeat but also a three-dimensional repeat [10]. Recently it has been demonstrated that most human HLA alleles do not bind mucin peptides, and thus MHC-restricted recognition of this epitope does not take place [8]. This makes the tandem

repeat especially attractive as a possible target for immunotherapy [2].

Monoclonal antibodies that react selectively with cancer cells offer the means for targeted delivery of diagnostic radioisotopes or therapeutic agents. But whole antibodies are large molecules and therefore have been found to have poor tumour penetration. Targeting with scFv antibody fragments [4] may overcome some of the limitations. scFv antibody fragments penetrate tumours better than IgG [30], are cleared more rapidly from the circulation, and provide greater targeting specificity [21]. The use of murine mAbs for repetitive therapy in humans has limitations because of the human anti-mouse antibody response, which has hampered therapy with anti-MUC1 mouse mAbs [7].

For some diagnostic and immunotherapeutic approaches scFv antibody fragments may not be the ideal format, e.g. because of their fast clearance rate [21]. However, depending on the target, the kind of therapy and the characteristics of the specific scFv, current molecular biotechnology techniques make it feasible to create a whole panel of antibody fragments: scFv, Fab, diabody, bispecific antibody fragments, minibody and full-size antibody [15], all of which carry the same specificity as the original antibody fragment from which they were derived.

We applied phage display technology to generate human scFv antibody fragments against the MUC-1 core protein by selecting a human antibody repertoire of 10^9 diversity [26] directly on the adenocarcinoma breast cancer cell line MCF-7 [6]. In phage display, peptides or proteins are expressed on the surface of phage as fusion proteins [27]. This allows the selection and amplification of phage clones with specific binding activities. Until now, antigen-specific scFv antibody fragments have been selected from murine [23], naive human [20], immune human [9], semi-synthetic [25] or synthetic [17], antibody gene repertoires displayed on the surface of bacteriophage [22].

Although phage display is a powerful way of selecting ligands against purified target proteins, it is less effective for selecting functional ligands for complex targets like living cells [18]. However, we show here that it is possible to screen a human phage display library for functional ligand binding on living tumour cells.

Material and methods

Cell lines

Human breast cancer cell lines MCF-7, ZR-75–1, MDA MB231 and SKBR3, the colon carcinoma cell line LS174T and the cell lines CHO (Chinese hamster ovarian cells) and HEK 293 (human embryonic kidney cells) were purchased from the American Type Culture Collection (Bethesda, Md.). The human endometrial cancer cell line HEC-1 was obtained from Dr. Morgane, Bomsel Faculty of Medicine, Reproductive Biology Laboratory, Paris, France. The cell lines were maintained in RPMI 1640 (Gibco BRL), supplemented with 10% FCS at 37 °C with 5% CO_2.

MUC-1 synthetic peptides

The MUC-1 peptides (Table 1) consisted of one (20 aa) and two (40 aa) of the 20-amino-acid MUC-1 tandem repeat and were synthesised by the Biochemistry Institute, University Lausanne, Switzerland.

The biotinylated peptide APDTRPAPGSTAPPAHGVTSK-ε-biotin was purchased from Anaspec, San Jose, USA.

The peptide was also presented on the surface of the hepatitis B core particle (HBcAg/MUC-1) in two different ways, termed N5 and N18: the difference between them being the position of the hydrophilic region of the 20-amino-acid tandem repeat (in N18 the PDTRP epitope is placed internally).

All peptides were used at a concentration of 10 μg/ml in PBS.

Mouse monoclonal antibodies

The mouse monoclonal antibodies NCL-MUC-1, which recognises a carbohydrate epitope of the MUC-1 glycoprotein (IgGCh), and NCL-MUC-1-CORE (IgGcore), which recognises a hexapeptide (TRPAPG) in the tandem repeat region of the MUC-1 core protein, were purchased from Readysysteme and used at a dilution of 1:50 in 1% BSA/PBS. The mouse monoclonal antibody SM3 [13], which recognises a pentapeptide (PDTRP) in the tandem repeat region of the MUC-1 core protein, was used at a concentration of 10 μg/ml in 1% BSA/PBS.

Propagation of phage from the human scFv phage display library

One litre of 2 × TY, 100 μg/ml ampicillin, 1% glucose was inoculated with an aliquot of the scFv library (10^9 diversity) [26] glycerol stock. The rescue of the phage was carried out as described in Marks et al. [19].

Cell surface selection

A human scFv phage display library of 10^9 diversity [26] was selected on the human breast cancer cell line MCF-7. Cells were cultured to sub-confluency (forming a monolayer) in 60 × 15-mm Petri dishes over night. The cells were washed with PBS and blocked for 2 h at room temperature with 2% Marvel/PBS. Then, 10^{12} t.u. of rescued library phage in 2 ml of 2% Marvel/PBS were added for 1 h at room temperature on a rocking platform. The dishes were washed 20 times with PBS and the cells were lysed with 100 μl of 100 mM triethylamine followed by neutralisation with 100 μl of 1 M Tris-HCl (pH 7.4). Half of the eluted phage was used to infect 10 ml of exponentially growing *E. coli* TG1 for 30 min at 37 °C before plating on TYE plates containing 100 μg/ml ampicillin and 1% glucose and incubation at 37 °C over night.

Soluble expression of scFv

After the third round of selection the eluted phages were used to infect 10 ml of exponentially growing *E. coli* HB2151, for 30 min at

Table 1 Presentation of the MUC-1 core protein. The amino acids (*aa*) thought to present an immunodominant epitope are underlined

	VNTR
MUC-1 20 aa	SA<u>PDTRP</u>APGSTAPPAHGVT
MUC-1 40 aa	SA<u>PDTRP</u>APGSTAPPAHGVT
	SA<u>PDTRP</u>APGSTAPPAHGVT
HbcAg N5	SA<u>PDTRP</u>APGSTAPPAHGVT (200 copies)
HbcAg N18	AHGVTSA<u>PDTRP</u>APGSTAPP (200 copies)
MUC-1 biotin	SA<u>PDTRP</u>APGSTAPPAHGVTK-ε-biotin

37 °C, before plating on TYE plates containing 100 μg/ml ampicillin and 1% glucose, followed by incubation at 37 °C overnight. Ninety-six single ampicillin-resistant colonies were picked and induced with 1 mM final concentration of isopropyl *β*-D-thiogalactopyranoside (IPTG) for the production of soluble scFv according to Marks et al. [19].

ELISA

The ELISA was carried out essentially as described by Ward et al. [28], except that the assay was developed with 3,3′,5,5′ tetramethylbenzidine (TMB) (Sigma). Reactions were stopped by the addition of H_2SO_4 after 20 min and readings taken at the OD_{450nm}.

The scFv antibody fragments were either tested at a concentration of 1 μg per well or 100 μl per well of overnight-induced culture.

The MUC-1 core peptide was coated at a concentration of 10 μg/ml in PBS, 100 μl/well overnight at 4 °C. The control antigens, human serum albumin (HSA), thyroglobulin and carcino embryonic antigen (CEA), for the specificity ELISA, were coated in the same way. The biotinylated peptide (10 μg/ml) was captured on streptavidin (10 μg/ml) coated wells, after blocking with 2% Marvel/PBS.

For the cell ELISA, cells were grown to approximately 80% confluency in flat-bottomed 96-well culture plates (Corning). The cells were washed with PBS and fixed for 10 min in 0.25% glutaraldehyde (Sigma).

For the epitope ELISA, the MUC-1 epitope PDTRP was expressed on the surface of filamentous phage. It was then coated onto solid phase and the ELISA performed as described above.

Inhibition studies with soluble MUC-1 core protein

The scFv (1 μg) was incubated for 1 h with soluble MUC-1 core protein, or negative control peptide, at varying concentrations for 30 min. The detection of bound scFv was performed as described above.

Fluorescence-activated flow cytometry

The scFv (10 μg) or mouse mAb was incubated with approximately 10^5 cells. scFv antibody fragments were detected with the murine monoclonal antibody 9E10, which recognised the C-terminal *myc* peptide tag [24], followed by an anti-mouse IgG Fc-specific FITC-conjugated antibody (Sigma). The fluorescence intensity of 10,000 events was analysed with a flow cytometer (FACScan, Becton Dickinson) and the mean fluorescence intensity was determined on a log scale. All incubations were carried out on ice for 30 min. Every incubation step was followed by a wash step, using ice-cold PBS.

Western blot

Two μg of purified scFv antibody fragments were run on a 10% SDS-PAGE gel and electroblotted onto nitro-cellulose. Filters were blocked for 1 h at room temperature in 10% Marvel/PBS. The scFv antibody fragments were detected with the murine monoclonal antibody 9E10 followed by anti-mouse IgG Fc-specific horseradish peroxidase conjugate (Sigma). Horseradish peroxidase was visualised with 3,3′diaminobenzidine tablets (Sigma) in the presence of cobalt ions.

Indirect immunofluorescence

The indirect immunofluorescence assays were performed on ethanol fixed human cell lines. The cells were incubated with 10 μg purified mAb or scFv; bound scFv antibody fragments were detected with the 9E10 antibody and visualised with an anti-mouse IgG Fc-specific FITC conjugate (Sigma). All incubations were performed for 1 h at room temperature. Washing steps were performed after each incubation with PBS. The slides were mounted with Immunofluore mounting medium (ICN Biomedicals) and photographed with a Zeiss Axioskop immunofluorescence microscope. The same experiment was carried out with the scFv incubated in human serum.

scFv stability in human serum

Two μg of purified scFv alone, or incubated with an excess of human serum (30 μl), at 4 °C or 37 °C for up to 24 h, were run on a 10% SDS-PAGE gel. The western blot was carried out as described. The same samples were also used to detect binding activity after incubation in human serum in indirect immunofluorescence assays, as described above.

Sequencing of DNA

The number of unique clones was determined by PCR amplification of the scFv insert, using the primers LMB3 (5′ CAG GAA ACA GCT ATG AC 3′) and fdSeq 1 (5′ GAA TTT TCT GTA TG/AG GG 3′), followed by digestion with the restriction enzyme *Bst*N1 (New England Biolabs). The variable (V) region genes from two clones of each restriction pattern were sequenced, using PCR cycle sequencing reactions with infrared-labelled primer according to the manufacturer's instructions (Licor). Sequencing reactions were analysed on a Licor automated DNA sequencer (4000L). Sequence analysis was performed using Sequencher 3.1 (Gene Codes). The sequences of the V_H and V_L genes were compared with the germline sequences in the V BASE sequence directory (Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK).

Subcloning of selected scFv

ScFv *V* genes were PCR amplified and digested with the restriction enzymes *Sfi*I and *Not*I (New England Biolabs),according to the manufacturer's instructions. They were then purified and subcloned into an expression vector containing the *myc* tag and hexa-histidine tag at the C-terminal end of the scFv [1].

Periplasmic preparation

Bacterial clones were cultured in 1 l of 2 × TY, 100 μg/ml ampicillin, 0.1% glucose and induced with 0.5 mM final concentration of IPTG for 3 h at 30 °C. The scFv antibody fragments were harvested from the periplasm, essentially as in Breitling et al. [5]. The periplasmic and osmotic shock fractions were pooled, and then dialysed against PBS. PMSF was added to a final concentration of 1 mM.

Purification of scFv

The scFv antibody fragments were purified by IMAC as described in Griffiths et al. [14]. The concentration was determined spectrophotometrically, assuming that A_{280} of 1 corresponded to a scFv concentration of 0.7 mg/ml.

Gelfiltration of scFv

A Sephadex 200 column (Pharmacia) was equilibrated with PBS. Human scFv antibody fragments were loaded and passed through at 1 ml/min. Aprotinin (6,500 Da), cytochrome C (12,400 Da), carbonic anhydrase (29,000 Da), BSA (66,000 Da) and dextran blue (2,000,000 Da) were run as molecular weight standards (Fluka).

Results

Selection of human scFv MUC-1

Human scFv antibody fragments against the cancer antigen MUC-1 were selected directly on the human breast cancer cell line MCF-7. After three rounds of selection, 96 individual bacterial clones were induced to produce soluble scFv. These scFv antibody fragments were tested in ELISA for their ability to bind to the MCF-7 cell line and the MUC-1 core protein. The core protein was either displayed as one 20-amino-acid tandem repeat, two tandem repeats or expressed on the hepatitis core particle (Table 1). Forty-eight per cent (46/96) of the scFv antibody fragments bound to the MCF-7 cell line. Of these, 25% (12/46) recognised the MUC-1 core protein. Six scFv antibody fragments that showed reactivity with the MUC-1 core protein, were chosen for further characterisation.

Sequence analysis of human scFv MUC-1

The variable genes encoding these scFv antibody fragments were PCR amplified and the product digested with the restriction enzyme *Bst*N1. Six scFv clones with unique *Bst*N1 fingerprints were chosen for further characterisation by sequencing. The variable domains of their heavy chains were all encoded by the VH3 family, germline DP 47. Their complementarity determining regions (CDR3) encoded seven amino acids, with the exception of scFv M9, which encoded 12 amino acids. The variable domains of the light chains were encoded by the Vλ3 family, germline DPL16, except for clone M2, which was encoded by the Vκ1 family, germline HK137. Although the *V* genes were encoded by the same family, they showed different levels of mutations.

Binding of human scFv MUC-1 to cell lines and specificity

The binding ability to cell lines was determined by FACS analysis, indirect immunofluorescence and ELISA. The result for binding to the MCF-7 cell line in FACS is shown in Fig. 1. The binding was also detected in indirect immunofluorescence (Fig. 2a) and, as examples, also on the MUC-1 expressing breast cancer cell line ZR75–1 and the colon carcinoma cell line LS174 for the human scFv M12, but not on the human embryonic kidney cell line HEK 293 (Fig. 2b). The human scFv antibody fragment also bound to the MUC-1 expressing cell lines ZR-75-1, SKBR3 and HEC-1, but not to the

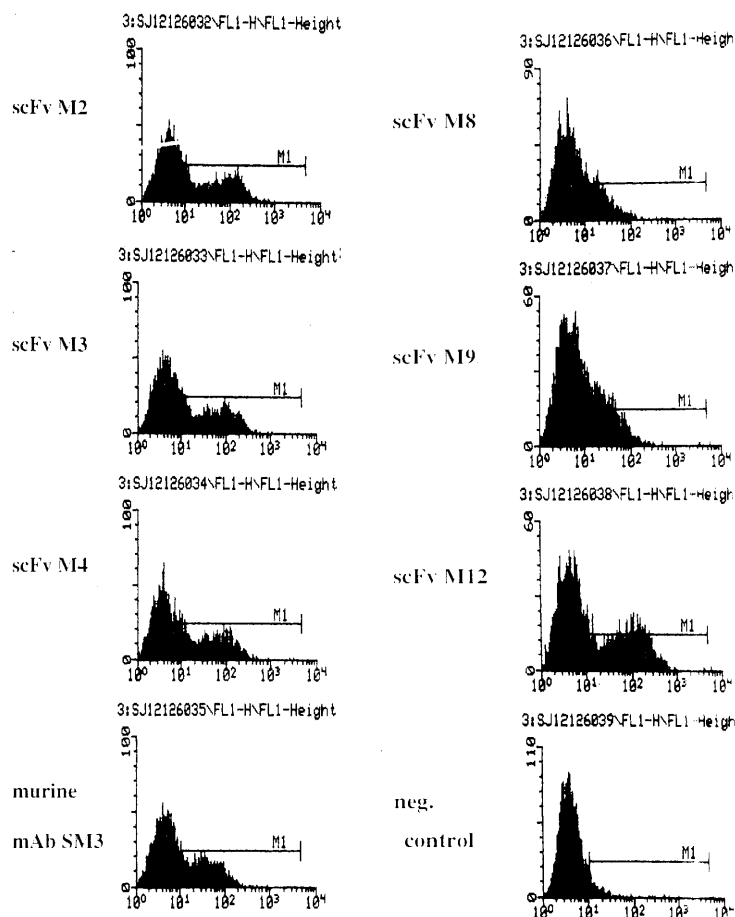


Fig. 1 FACS analysis of human scFv MUC-1 on the human breast cancer cell line MCF-7. Bound human scFv was detected with the mAb 9E10, which recognised the *myc* tag, followed by an anti-mouse mAb conjugated with FITC. The negative control was a human scFv recognising a different antigen. The positive control was the murine mAb SM3, which recognised the MUC-1 core protein

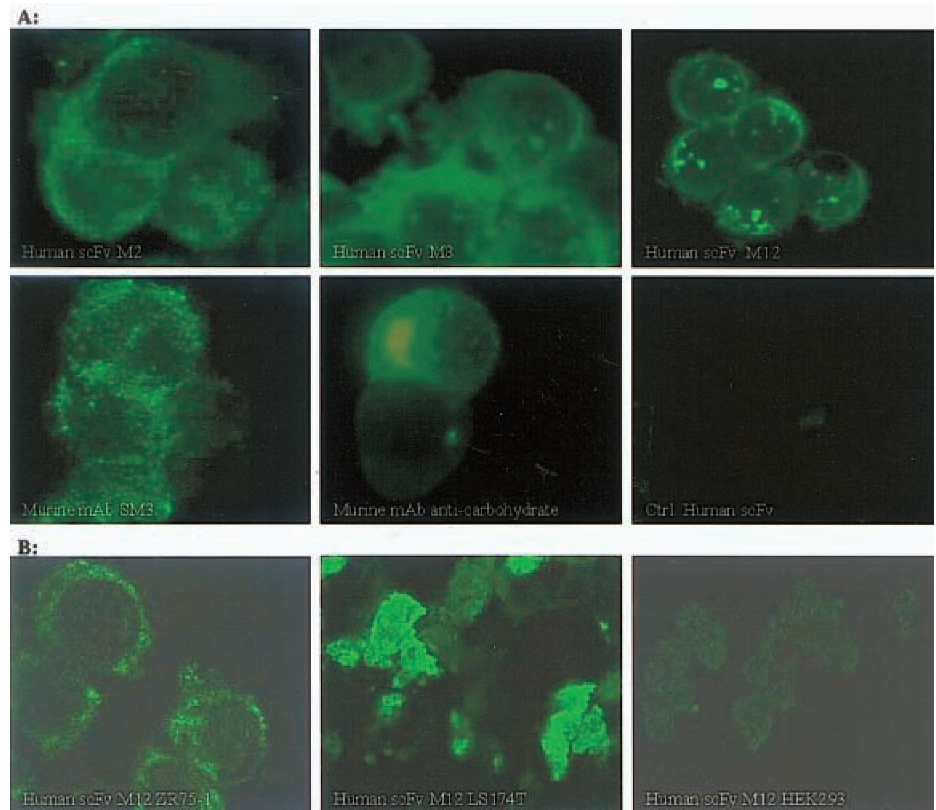


Fig. 2 **a** Immunofluorescence of human scFv MUC-1 on the ethanol-fixed human breast cancer cell line MCF-7. Bound human scFv was detected with the mAb 9E10, which recognises the *myc* tag, followed by an anti-mouse mAb conjugated with FITC. The negative control was a human scFv recognising a different antigen. The positive control was the murine mAb SM3, which recognised the MUC-1 core protein, and the murine mAb NCL-MUC-1, which recognised a carbohydrate epitope of the MUC-1 glycoprotein. **b** Immunofluorescence of human scFv M12 on ethanol fixed human breast cancer cell line ZR75-1, the human colon carcinoma cell line LS174T and the human embryonic kidney cell line HEK 293. Bound human scFv was detected with mAb 9E10, followed by anti-mouse mAb::-FITC, as in **a**. The human scFv M12 stained positive on human carcinoma cell lines ZR75-1 and LS174T

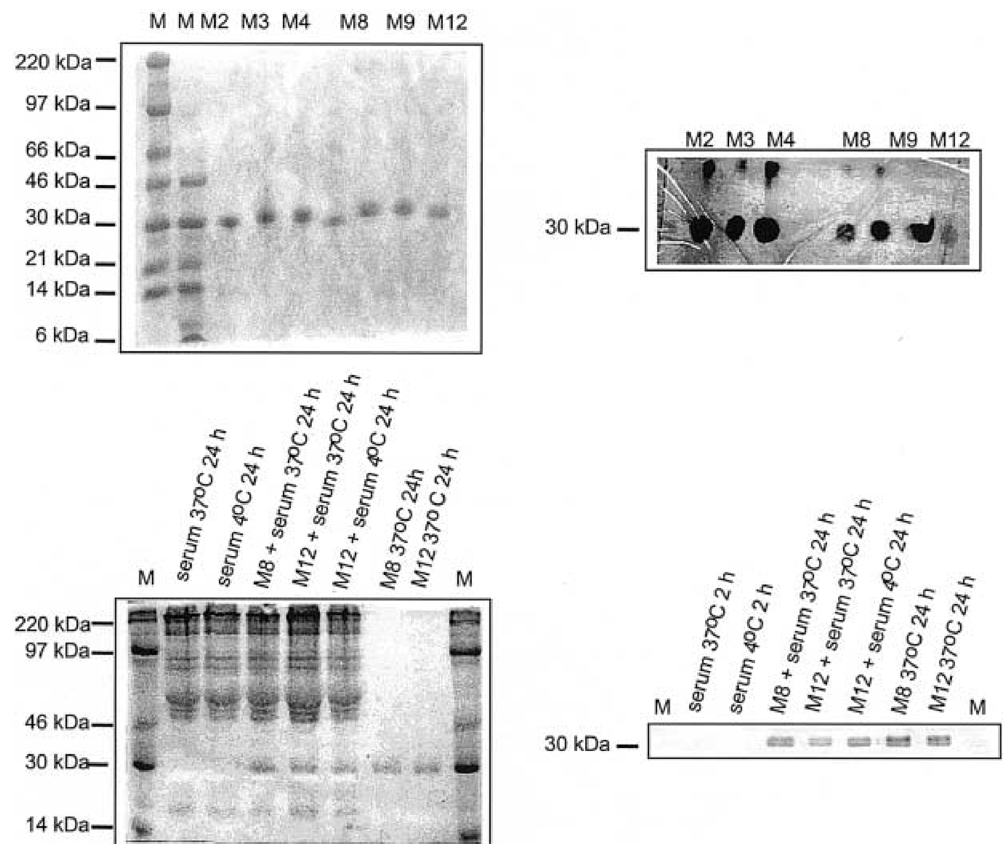


Fig. 3 *Top panel*: Purification of human scFv MUC-1. Two µg of purified scFv antibody fragments were run on a 10% SDS-PAGE gel and either stained with Coomassie blue (*left*) or transferred to nitrocellulose (*right*) and detected with mAb 9E10, which recognises the *myc* tag, followed by an HRP-conjugated anti-mouse mAb and visualisation with DAB. *Bottom panel*: Incubation of human scFv MUC-1 with human serum. Two µg of purified scFv antibody fragments were incubated with an excess of human serum (30 µl) for 24 h at different temperatures and run on a 10% SDS-PAGE gel and either stained with Coomassie blue (*left*) or transferred to nitrocellulose and detected with mAb 9E10, anti-mouse mAb::HRP and DAB, as before

control cell lines CHO and HEK 293, as shown by ELISA. ELISA on the MUC-1 core protein and negative control proteins further confirmed specificity. The purified scFv were visualised on a Coomassie-stained SDS-PAGE and detected on a western blot with a mAb recognising the *myc* tag (Fig. 3 top panel).

Subcloning and purification of human scFv MUC-1

The scFv-encoding DNAs were subcloned into a phagemid vector containing the six-histidine tag for subsequent purification on IMAC. One-litre cultures were induced and the scFv antibody fragments purified from the periplasm. The yields varied between 6 and 10 mg/l. To determine whether the scFv antibody fragments were monomeric (29 kDa) or formed multimers, they were passed through a sizing column. All showed one single monomer peak at 29 kDa (Fig. 4 shows the gel filtration of the human scFv M12).

Stability of human scFv MUC-1 in human serum

As these scFv antibody fragments might be used in humans, their stability in human serum is of great interest. For this reason, the scFv antibody fragments were incubated with human serum at 4 °C and 37 °C, followed by SDS-PAGE and western blot analysis (Fig. 3 bottom panel), and also indirect immunofluorescence (data not shown). All of the scFv antibody fragments still recognised the MUC-1 expressing cells, confirming their biological activity after incubation with human serum.

Inhibition of human scFv MUC-1

It was possible to inhibit the binding of the scFv antibody fragments M3, M4 and M9 to the MUC-1 core protein on solid phase by the addition of soluble MUC-1 core protein in varying concentrations, but not by the addition of a negative control peptide (Fig. 5)

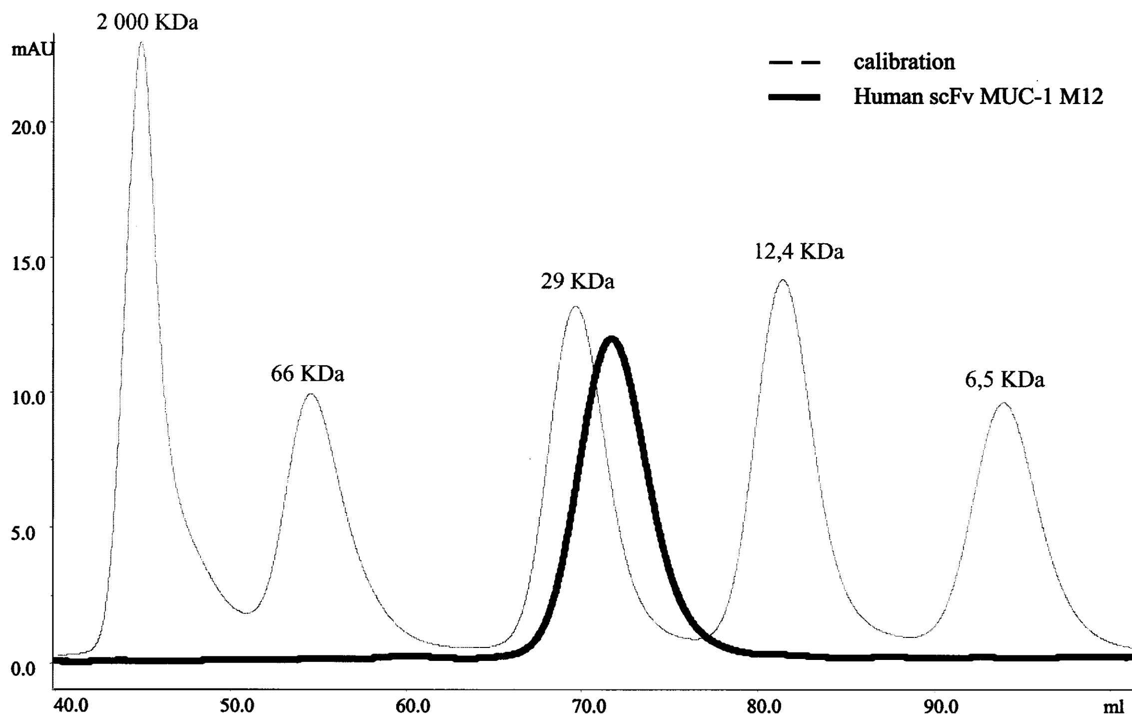
Epitope analysis of human scFv MUC-1

As the epitope PDTRP, which is recognised by the mAb SM3 and others, is thought to be an immunodominant region, the reactivity of the selected human scFv to this epitope was tested in ELISA. Figure 6 shows that the scFv antibody fragments M2, M3, M8 and M12, and the mAb SM3, recognised this epitope. In contrast, scFv antibody fragments M4 and M9 might recognise a different region of the MUC-1 tandem repeat.

Discussion

Several advances made during the past years will probably facilitate the development of therapeutic antibodies. Phage libraries displaying antibody fragments are the fastest route to obtaining human antibody

Fig. 4 Gel filtration of human scFv M12 on a Sephadex 200 column. The column was calibrated between 6.5 kDa and 2,000 kDa. The human scFv shows a single peak at approx. 28 kDa (= monomer)



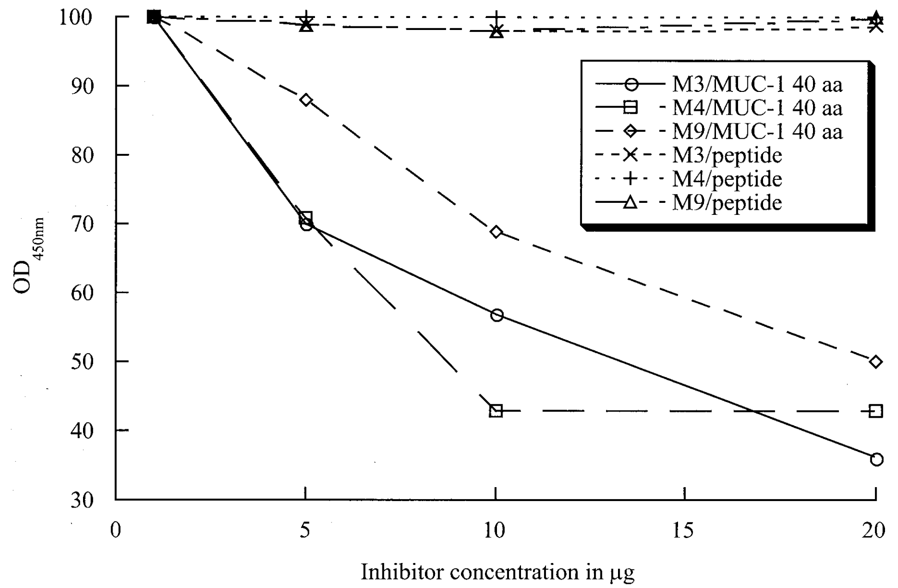


Fig. 5 Inhibition of binding of human scFv anti-MUC-1 to the MUC-1 core protein by the addition of soluble MUC-1 core protein. The scFv antibody fragments were allowed to bind first to the MUC-1 core protein on solid phase and were then displaced by soluble MUC-1 core protein. An unrelated peptide (20 aa) was used as the control. Bound human scFv antibody fragments were detected with the mAb 9E10, which recognised the myc tag, followed by an anti-mouse mAb conjugated with HRP and subsequent treatment with DAB

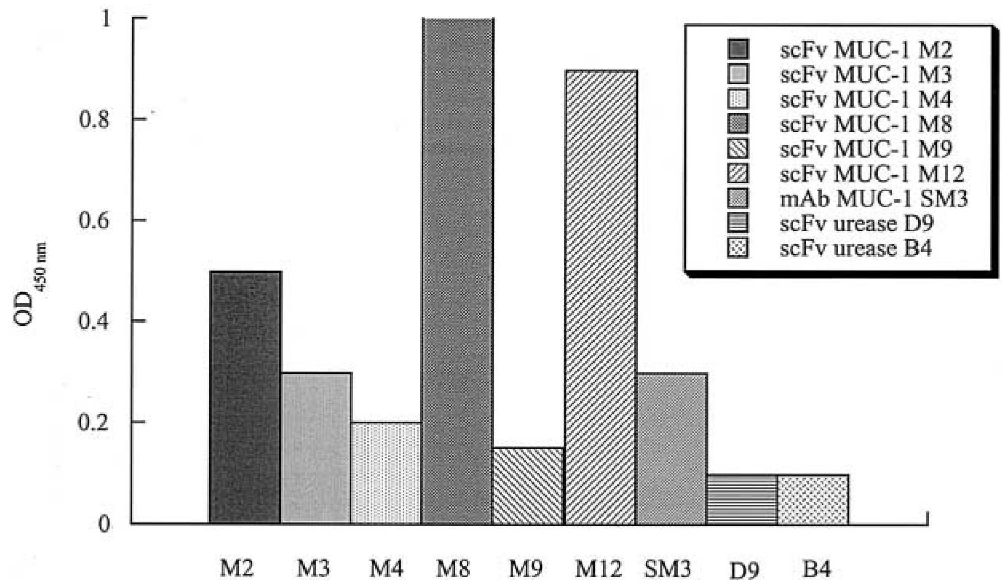


Fig. 6 ELISA on the PDTRP epitope of MUC-1 expressed on phage. Bound human scFv antibody fragments were detected with the mAb 9E10, which recognises the *myc* tag, followed by an anti-mouse mAb::HRP conjugate and DAB treatment. scFv urease B4 and D9 were the negative controls. Murine mAb SM3, which recognised the MUC-1 core protein, was the positive control

fragments. In addition, the phage display format is ideal for optimisation of the isolated variable domains.

So far, selections of *V* gene phage display libraries have mainly been carried out on immobilised purified antigen or on purified antigen in solution. To find other markers on tumour cells that might be suitable targets in immunotherapy, it will be necessary to select binders directly on tumour cell lines or tumour tissue.

Our results demonstrate that human scFv antibody fragments against the tumour-associated cell surface antigen MUC-1 can be produced via selection from a phage antibody repertoire on living cells. For a specific antigen, the success of this approach depends upon the presence of a scFv binder in the library and the ability to select it preferentially against a background of the many other antigens present on the cell surface. This will depend on the relative binding affinities of the phage, and, in turn, on the relative affinity of each scFv for the antigen, the number of copies displayed on the phage, the surface density of antigen on the cell and its accessibility to the bulky phage. It was possible to select 12 different monomeric scFv on the breast cancer cell line MCF-7 that did react with the tandem repeat of the MUC-1 core protein. Six of these scFv antibody fragments were characterised with respect to their binding specificity to MUC-1 on synthesised peptides (1 and 2 tandem repeats), on the tandem repeat presented in two different variants on the hepatitis B core particle, on the biotinylated peptide and on different adenocarcinoma cell lines. The scFv antibody fragments could be detected by

ELISA, FACS and indirect immunofluorescence. Their stability in human serum at 37 °C was demonstrated ex vivo. However, their pharmacokinetics will have to be investigated in vivo. The selected human scFv antibody fragments not only recognised the MUC-1 core protein on the breast cancer cell line MCF-7 on which they were selected, but also on other MUC-1 expressing breast carcinoma, colon carcinoma and endometrial cancer cell lines. For imaging or targeting of tumours expressing MUC-1 it might be feasible to use these human scFv antibody fragments, or second generation multivalent derivatives, as vehicles to deliver (for example) radioactivity, cytotoxic drugs or secondary signals for T-cell activation to the tumour site.

The ability of the human scFv antibody fragments to bind to the mucin MUC-1 on carcinoma cell surfaces will allow their potential as a diagnostic and therapeutic reagent of clinical utility to be investigated.

Acknowledgements The human scFv anti-urease D9 and B4 were a gift from Mehdi Houimel. The human endometrial cancer cell line HEC-1 was obtained from Dr. Morgane, Bonsel Faculty of Medicine, Reproductive Biology Laboratory, 75674 Paris and the murine monoclonal antibody SM3 was obtained from Dr. Taylor-Papadimitriou, ICRF, London. The MUC-1 peptide presented on the surface of the hepatitis B core particle was a kind gift from Dr. Arya Biragyn, National Cancer Institute, Frederick, Maryland. This work was supported by the Swiss National Science Foundation grant No. 31-49361.96 and by the Ligue Neuchâteloise contre le cancer.

References

1. Amersdorfer P, Wong C, Chen S, Smith T, Deshpande S, Sheridan R, Finnern R, Marks JD (1997) Molecular characterisation of murine humoral immune response to botulinum neurotoxin type A binding domain as assessed by using phage antibody libraries. Infect Immun 65: 3743
2. Barratt-Boyes SM (1996) Making the most of mucin: a novel target for tumor immunotherapy. (review) Cancer Immunol Immunother 43: 142
3. Berry N, Jones DB, Smallwood J, Taylor I, Kirkham N, Taylor-Papadimitriou J (1985) The prognostic value of the monoclonal antibodies HMFG1 and HMFG2 in breast cancer. Br J Cancer 51: 179
4. Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, Lee SM, Lee T, Pope SH, Riordan GS, Whitlow M (1988) Single-chain antigen-binding proteins. Science 242: 423
5. Breitling F, Duebel S, Seehaus T, Klewinghaus I, Little M (1991) A surface expression vector for antibody screening. Gene 104: 147
6. Brooks SC, Locke ER, Soule HD (1973) Estrogen receptor in a human cell line (MCF-7) from breast carcinoma. J Biol Chem 248: 6251
7. DeNardo SJ, Kramer EL, O'Donnell RT, Richman CM, Salako QA, Shen S, Noz M, Glenn SD, Ceriani RL, DeNardo GL (1997) Radioimmunotherapy for breast cancer using indium-111/yttrium-90 BrE-3: results of a phase I clinical trial. J Nucl Med 38: 1180
8. Domenech N, Henderson RA, Finn OJ (1995) Identification of an HLA-A11-restricted epitope from the tandem repeat domain of the epithelial tumor antigen mucin. J Immunol 155: 4766
9. Finnern R, Pedrollo E, Fisch I, Wieslander J, Marks JD, Lockwood CM, Ouwehand WH (1997) Human autoimmune anti-proteinase 3 scFv from a phage display library. Clin Exp Immunol 107: 269
10. Fontenot JD, Tjandra N, Bu D, Ho C, Montelaro RC, Finn OJ (1993) Biophysical characterization of one-, two-, and three-tandem repeats of human mucin (muc-1) protein core. Cancer Res 53: 5386
11. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, Pemberton L, Lalani EN, Wilson D (1990) Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 265: 15286
12. Girling A, Bartkova J, Burchell J, Gendler S, Gillet C, Taylor-Papadimitriou J (1989) A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary breast carcinoma. Int J Cancer 43: 1072
13. Granowska M, Biassoni L, Carroll MJ, Howell R, Mather SJ, Ellison D, Granowski A, Britton KE (1996) Breast cancer 99mTc SM3 radioimmunoscintigraphy. Acta Oncol 35: 319
14. Griffiths AD, Williams SC, Hartley O, Tomlinson IM, Waterhouse P, Crosby WL, Kontermann RE, Jones PT, Low NM, Allison TJ, Prospero TD, Hoogenboom HR, Nissim A, Cox JPL, Harrison JL, Zaccolo M, Gherardi E, Winter G (1994) Isolation of high affinity human antibodies directly from large synthetic repertoires. EMBO J 13: 3245
15. Hudson PJ (1999) Recombinant antibody constructs in cancer therapy. Curr Opin Immunol 11: 548
16. Jerome KR, Barnd DL, Bendt KM, Boyer CM, Taylor-Papadimitriou J, McKenzie IF, Bast RC Jr, Finn OJ (1991) Cytotoxic T-lymphocytes derived from patients with breast adenocarcinoma recognize an epitope present on the protein core of a mucin molecule preferentially expressed by malignant cells. Cancer Res 51: 2908
17. Krebs B, Griffin H, Winter G, Rose-John S (1998) Recombinant human single chain Fv antibodies recognizing human interleukin-6. Specific targeting of cytokine-secreting cells. J Biol Chem 273: 2858
18. Kupsch JM, Tidman NH, Kang NV, Truman H, Hamilton S, Patel N, Newton Bishop JA, Leigh IM, Crowe JS (1999) Isolation of human tumor-specific antibodies by selection of an antibody phage library on melanoma cells. Clin Cancer Res 5: 925
19. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G (1991) By-passing immunization. Human antibodies from *V*-gene libraries displayed on phage. J Mol Biol 222: 581
20. Marks JD, Ouwehand WH, Bye JM, Finnern R, Gorick BD, Voak D, Thorpe S, Hughes-Jones NC, Winter G (1993) Human antibody fragments specific for human blood group antigens from a phage display library. Biotechnology 11: 1145
21. Mayer A, Chester KA, Flynn AA, Begent RH (1999) Taking engineered anti-CEA antibodies to the clinic. J Immunol Methods 231: 261
22. McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348: 552
23. Muller BH, Lafay F, Demangel C, Perrin P, Tordo N, Flamand A, Lafaye P, Guesdon JL (1997) Phage-displayed and soluble mouse scFv fragments neutralize rabies virus. J Virol Methods 67: 221
24. Munro S, Pelham HRB (1986) An hsp70-like protein in the ER: identity with the 78kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell 46: 291
25. Nissim A, Hoogenboom HR, Tomlinson IM, Flynn G, Midgley C, Lane D, Winter G (1994) Antibody fragments from a 'single pot' phage display library as immunochemical reagents. EMBO 13: 692
26. Sheets MD, Amersdorfer P, Finnern R, Sargent P, Lindqvist E, Schier R, Hemingsen G, Wong C, Gerhart JC, Marks JD (1998) Efficient construction of a large nonimmune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens. Proc Natl Acad Sci USA 95: 6157

27. Smith GP (1988) Filamentous phages as cloning vectors. (review) Biotechnology 10: 61
28. Ward S, Guessow D, Griffiths AD, Jones PT, Winter G (1989) Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*. Nature 341: 544
29. Wreschner DH, Hareuveni M, Tsarfaty I, Smorodinsky N, Horev J, Zaretsky J, Kotkes P, Weiss M, Lathe R, Dion A, Keydar I (1990) Human epithelial tumor antigen cDNA sequences. Differential splicing may generate multiple protein forms. Eur J Biochem 189: 463
30. Yokota T, Milenic DE, Whitlow M, Schlom J (1992) Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res 52: 3402