Selection of Mimotopes of the Cell Surface Adhesion Molecule Mel-CAM from a Random pVIII-28aa Phage Peptide Library

Christine Hafner,*‡¶ Ursula Samwald,*† Stefan Wagner,†¶ Franco Felici,§ Elisabeth Heere-Ress,* Erika Jensen-Jarolim,† Klaus Wolff,*¶ Otto Scheiner,†¶ Hubert Pehamberger,*¶ and Heimo Breiteneder†¶

*Department of Dermatology, Division of General Dermatology, University of Vienna, Austria; †Department of Pathophysiology, Division of Applied Experimental Pathology, University of Vienna, Austria; ‡Department of Clinical Pharmacology, University of Vienna, Austria; §Department of Microbiology, University of Messina, Italy, and Kenton Laboratories, Pomezia, Italy; ¶CLEXO Center of Excellence in Clinical and Experimental Oncology, General Hospital Vienna, Vienna, Austria

The cell surface adhesion molecule Mel-CAM is highly expressed in advanced primary and metastatic melanoma. Mel-CAM was first described as an integral membrane glycoprotein of malignant melanoma cells. The murine monoclonal antibody MAd18-5D7 recognizes an epitope of the extracellular domain of Mel-CAM and is able to enhance Mel-CAM mediated adhesion of melanoma cells in aggregation assays. For the characterization of peptides that antigenically mimic surface-exposed areas of Mel-CAM we screened a newly constructed random pVIII-28aa bacteriophage peptide library against MAd18-5D7. After three panning rounds a population of phages binding to MAd18-5D7 was enriched. Peptides expressed on the surface of these phages were then tested for their specificity for the antibody's antigen binding site. DNA sequences coding for two specific peptide ligands were determined. One of the deduced amino acid sequences showed similarity to a portion of the sequence of the third immunoglobulin-like extracellular domain of Mel-CAM. Both peptides blocked the interaction of MAd18-5D7 with Mel-CAM present in a MelJuSo melanoma cell line lysate. Phage displayed as well as synthetic peptides inhibited in a dose-dependent manner the binding of MAd18-5D7 to recombinant Mel-CAM in enzyme-linked immunosorbent assay experiments. No such inhibition was observed using a panel of other anti-Mel-CAM antibodies. Our results clearly indicate that these 28mer peptides are structural equivalents of the MAd18-5D7 epitope of Mel-CAM and that they will be useful tools for further *in vitro* and *in vivo* studies of Mel-CAM mediated cell–cell interaction.
Key words: MAd18-5D7/Mel-CAM/mimotope/MUC18/phage display. J Invest Dermatol 119:865–869, 2002

Mel-CAM (also known as MUC18, MCAM, or CD146) displays significant homology to cell adhesion molecules containing the characteristic V-V-C2-C2-C2 immunoglobulin-like domain structure (Holness and Simmons, 1994). The complete coding region of Mel-CAM was originally cloned and sequenced from a human melanoma cDNA library screened with the monoclonal antibody MUC18 (Lehmann *et al*, 1989). Mel-CAM is an integral membrane glycoprotein with a molecular mass of 113 kDa and contains several protein kinase recognition motifs in its cytoplasmic domain, suggesting an involvement in cell signaling (Shih, 1999). Mel-CAM is strongly upregulated in melanomas compared to benign lesions and is expressed by more than 80% of metastatic lesions (Kraus *et al*, 1997). It is also found on normal endothelium among several other tissue types (Shih, 1999), e.g., on activated T cells (Pickl *et al*, 1997), smooth muscles (Shih *et al*, 1994), and vascular endothelial cells (Bardin *et al*, 1996; St Croix *et al*, 2000). Its function for these cells is unclear. For melanoma cells recent data suggest that Mel-CAM is involved in cell–cell adhesion (Satyamoorthy *et al*, 2001). The expression of Mel-CAM on melanoma cells correlates with an invasive phenotype indicating its use as a cell surface marker of metastasis formation and tumor progression (Xie *et al*, 1997). Mel-CAM has been shown to be involved in tumor–endothelial cell interaction that might be part of the extravasation process of tumor cells (Johnson *et al*, 1997; Shih *et al*, 1997). To date the ligand mediating heterophilic aggregation remains unidentified (Shih *et al*, 1997). It has been reported that activation of Mel-CAM leads to tyrosine phosphorylation of $p125^{FAK}$ and of paxillin and is associated with $p59^{fyn}$ (Anfosso *et al*, 1998).

The use of bacteriophage display peptide libraries greatly facilitates identifying peptide ligand-binding sites for proteins of interest that are difficult to identify by conventional techniques. Smith *et al* pioneered bacteriophage library construction in which vectors are constructed for expression of random peptides fused to phage coat proteins, e.g. pIII or pVIII of filamentous phage (Smith, 1985; reviewed by Zwick *et al*, 1998). Peptide sequences that bind to a wide range of ligands such as monoclonal antibodies, receptors, and carbohydrate moieties have been successfully identified (Moe *et al*, 1999; Ruoslahti, 2000). We applied bacteriophage display

Manuscript received March 28, 2002; revised June 17, 2002; accepted for publication July 8, 2002

Reprint requests to: Heimo Breiteneder, Ph.D., Department of Pathophysiology, Vienna General Hospital, Waehringer Guertel 18–20, A-1090 Vienna, Austria; Email: heimo.breiteneder@akh-wien.ac.at

technology to define structural equivalents (mimotopes) of the cell adhesion molecule Mel-CAM that mimic its epitope of MAd18-5D7.

MATERIALS AND METHODS

Antibodies and phage display peptide library The pVIII-28aa phage display peptide library is composed of random 28 amino acid residues, displayed on filamentous phage as fusion to the NH_2-terminus of the major coat protein pVIII. The library has been generated essentially as described previously, by cloning an oligonucleotide containing 28 randomized codons (27 NNS and one NNG triplet) in the pC89 phagemid vector (Felici *et al*, 1991). Library complexity as derived from the number of individual clones obtained upon bacterial transformation was about 1×10^8.

The anti-Mel-CAM monoclonal antibodies MUC BA18.3 and MUC18 were a gift of J. P. Johnson (Institute of Immunology, University of Munich, Munich, Germany) and are described elsewhere (Lehmann *et al*, 1987; 1989). MAd18-5D7 is a mouse IgM monoclonal antibody (MoAb) and was selected on the basis of its functional activity of enhancing Mel-CAM mediated adhesion in aggregation assays (J. P. Johnson, Institute of Immunology, University of Munich, Munich, unpublished results). The MoAbs MUC18 and MUC BA18.3 are mouse IgGs. MUC BA18.3 is directed against the cytoplasmic region of Mel-CAM (Lehmann *et al*, 1989). MUC18 recognizes an epitope of the extracellular portion of the Mel-CAM molecule (Lehmann *et al*, 1987). For isotype control the mouse IgM MoAb clones TEPC 183 and MOPC 104E as well as the human IgM MoAb I8260 were purchased from Sigma-Aldrich (St. Louis, MO).

Cell lines and recombinant Mel-CAM The human melanoma cell line MelJuSo (Lehmann *et al*, 1987) that expresses high levels of Mel-CAM and the melanoma cell line MD3a (Versteeg *et al*, 1989) with no detectable expression of Mel-CAM were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics (Gibco, Invitrogen, Carlsbad, CA). The recombinant extracellular Mel-CAM domain was a gift of M. Herlyn (The Wistar Institute, Philadelphia, PA) and is described elsewhere (Shih *et al*, 1997).

Selection of specific phages from the pVIII-28aa phage display peptide library For each round of selection polystyrene tubes (Nunc, Naperville, IL) were coated overnight at 4°C with 10 μg of the murine MoAb MAd18-5D7 per ml coating buffer (50 mM $NaHCO_3$, pH 9.4). Tubes were then blocked with blocking buffer (5 mg per ml dialyzed bovine serum albumin and 0.02% NaN_3 in 0.1 M $NaHCO_3$, pH 9.6) and washed extensively with phosphate-buffered saline (PBS), 0.1% Tween 20. Incubation was performed with 10^{12} phage previously preabsorbed on MoAbs TEPC183 and MOPC 104E at room temperature for 1 h. After washing with PBS/0.1% Tween 20, bound phages were eluted with glycin–HCl, pH 2.2, and neutralized with 1 M Tris–HCl, pH 9.1. Eluted phages were amplified by infecting *Escherichia coli* TG1 at an optical density at 600_{nm} of 1.5 and purified with a 20% PEG/2.5 M NaCl precipitation. Two further rounds of selection where performed against the antibody MAd18-5D7. After three rounds of selection colony screening was performed (Felici *et al*, 1991). Phages were purified from single colonies and tested for their reactivity with MAd18-5D7 by phage enzyme-linked immunosorbent assay (ELISA).

Third round phage pool analysis The pool of phages from the third selection round was analyzed by sequencing single phage clones. Phagemid DNA was purified from overnight cultures of single phage clones. DNA sequencing was performed by the Sanger dideoxy method using a pVIII specific fluorescent labeled primer (Leitner *et al*, 1998) and analyzed by a LI-COR DNA Sequencer 4000 L (LI-COR, Lincoln, NE).

Phage ELISA Ninety-six well microtiter plates (Maxisorp, Nunc) were coated with 2 μg anti-pIII antibody (Dente *et al*, 1994) per ml coating buffer overnight at 4°C. Plates were blocked (5% skim milk and 0.05% Tween 20 in PBS, pH 7.3) for 2 h at room temperature and washed (0.05% Tween 20 in PBS, pH 7.3). Purified phages were diluted in blocking buffer and plates were incubated with the dilutions for 3 h at room temperature. After further washing and incubating with 1 μg each MAd18-5D7, MUC BA18.3, MUC18, and 1 μg of an equimolar mix of TEPC 183 and MOPC 104E per ml blocking buffer, bound IgM or IgG was detected using AP-conjugated antimouse IgM (μ-specific) or IgG (μ-specific) (Sigma) followed by addition of p-nitrophenylphosphate (Sigma). The absorbance was measured at 405 nm.

Synthesis of peptides The peptides GRALPGTAEWTSYPGWTSR-LKNLEPAVG (mimotope 1) and RVKHMLVGASSRVPGFFSSLPGF-QGPFT (mimotope 2) and an unrelated peptide (ch3) were synthesized by piCHEM (Graz, Austria). The purity of the peptides was greater than 95% as assessed by high performance liquid chromatography.

ELISA inhibition assay Ninety-six well microtiter plates (Maxisorp, Nunc) were coated overnight at 4°C with 1 μg recombinant Mel-CAM per ml coating buffer (50 mM $NaHCO_3$, pH 9.6). One microgram MAd18-5D7 was preincubated with varying concentrations (10^3, 10^6, and 10^9) of purified phages overnight at 4°C and added to the blocked microtiter plates. Bound IgM was detected using AP-conjugated antimouse IgM (μ-specific) (Sigma) followed by addition of p-nitrophenylphosphate (Sigma). The absorbance was measured at 405 nm. As controls, wild-type phage wt pc89, phages displaying unrelated peptides not specific for MAd18-5D7, and the MoAbs TEPC 183, MOPC 104E, and I8260 (Sigma) as isotype controls were used in the above-mentioned concentrations. In an additional ELISA inhibition assay 0.5 μg MAd18-5D7 was preincubated with varying concentrations (1, 10, 100, and 500 μg) of synthetic peptides and then transferred to an ELISA plate coated with recombinant Mel-CAM. The assay was continued as described above.

Immunoblot and inhibition assay For Western blotting, MelJuSo and MD3a cells were lyzed in lysis buffer [50 mM Tris, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS)] to obtain protein lysates for immunoblotting experiments and adjusted to a protein concentration of 500 μg per ml. Proteins of MelJuSo and MD3a protein lysates were separated on an 8% polyacrylamide gel and transferred onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH). Five micrograms of recombinant Mel-CAM were electrophoresed on an 8% polyacrylamide gel and transferred onto a nitrocellulose membrane (Schleicher and Schuell). Antibodies MAd18-5D7 and MUC BA18.3 were preincubated with 5×10^9 purified phages overnight at 4°C and added to the blocked nitrocellulose membrane. Wild-type phage wt pc89 in the same concentration as the phages displaying peptides was used as control. Bound antibodies were visualized using an AP-conjugated antimouse IgM (μ-specific) (Sigma) or an AP-conjugated antimouse IgG (γ-specific), respectively, followed by equilibration in AP buffer (0.1 M Tris base, pH 9.5, 0.1 M NaCl, 1 mM $MgCl_2$) and development with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate-p-toluidine.

Phage proteins were electrophoresed in 15% SDS polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. Blocked membranes were incubated with the MoAbs MAd18-5D7, MUC18, MUC BA18.3, TEPC 183, MOPC 104E, and I8260 and their respective secondary antibodies and developed as mentioned above.

RESULTS

Identification of MAd18-5D7 specific ligands We screened a linear pVIII-28mer phage peptide library to identify ligands that are able to bind specifically to the antigen recognition site of the MoAb MAd18-5D7. Three rounds of biopanning using the MoAb MAd18-5D7 as a target were performed. Throughout these rounds the phage pool was enriched to 9×10^{11} phages per ml elution buffer. In colony screening 53 single clones reacted with the MoAb MAd18-5D7. A phage ELISA was performed to test the clones' specificity for MAd18-5D7. Phages that produced an OD_{405} greater than the mean ± 3SD of wt pc89 phage were considered to be specific for the antibody. Ten clones produced such an OD greater than the mean ± 3SD of wild-type phage wt pc89 (OD ± SD = 0.064 ± 0.004). These clones were regarded as specific for the antibody and subjected to DNA sequencing (data not shown).

Sequence analysis Single stranded DNA of the 10 phage clones that bound specifically to the MoAb MAd18-5D7 was purified and the inserts coding for the mimotopes were sequenced. Two different sequences were obtained. Eight phage clones displayed mimotope 1, and two clones displayed mimotope 2. By linear alignment using the BLAST search algorithm it was possible to align the sequence of mimotope 1 to the sequence of Mel-CAM between amino acid positions 307–310 (LEPA). For mimotope 2

Phage clone	Sequence	Frequency
1	GRALPGTAEWTSYPGWTSRLKNLEPAVG	80
2	RVKHMLVGASCRVPGFFCSLPGFQGPFT	20

Figure 1. Insert sequences of phages displaying mimotopes for MoAb MAd18-5D7. Frequency denotes the percentage of phage clones sequenced with identical inserts.

no linear alignment to the sequence of Mel-CAM could be observed. The deduced amino acid sequences are listed in **Fig 1**.

Phage ELISA A phage ELISA to confirm the specificity of the two selected mimotopes for the MoAb MAd18-5D7 was performed. Therefore, the two mimotopes were tested for binding to the MoAb MAd18-5D7 as well as to the control antibodies MUC BA18.3, MUC18, TEPC 183, and MOPC 104E. Both mimotopes only bound to the selecting antibody MAd18-5D7 (**Fig 2**).

Immunoblot Recombinant Mel-CAM and proteins of a MelJuSo lysate were electrophoresed and blotted onto nitrocellulose membrane. Strips were incubated with MAd18-5D7 or MUC BA18.3. A single band was visible at approximately 113 kDa for natural Mel-CAM with MAd18-5D7 or MUC BA18.3 (**Fig 3*a***). MAd18-5D7 reacted with the recombinant extracellular domain of Mel-CAM (**Fig 3*b***), whereas no band was observed in a lysate of the Mel-CAM negative cell line MD3a (data not shown).

Immunoblot inhibition To test phage displayed mimotopes 1 and 2 for their ability to inhibit the binding of MoAb MAd18-5D7 to Mel-CAM, the antibody was preincubated with 5×10^9 phages displaying mimotope 1 or 2 peptides, respectively, and then used to probe a MelJuSo lysate blotted to nitrocellulose strips. Both mimotopes were able to abolish the binding of MAd18-5D7 to Mel-CAM in the MelJuSo cell line lysate as well as to recombinant Mel-CAM (**Fig 3*a***, ***b***). No inhibition was detected when preincubating MAd18-5D7 with the unrelated phage displayed peptide ch3, obtained by panning the mouse MoAb BIP1 (Jarolim *et al*, 1989) with the 28mer library. No inhibition was observed when MAd18-5D7 was preincubated with wild-type phage wt pc89 (**Fig 3*a***, ***b***). No inhibition was observed when the MoAb MUC BA18.3 was preincubated with the phages displaying the peptides or when the preincubation was performed with wild-type phage wt pc89 (**Fig 3*a***). Immunoblots of SDS-PAGE separated phage proteins were used to demonstrate the specificity of binding. Specific IgM binding phages displaying peptide mimotopes 1 and 2 were subjected to 15% SDS-PAGE, blotted onto nitrocellulose, and incubated with either MAd18-5D7 or TEPC 183, MOPC 104E, I8260, and MUC18. A signal occurred for the phage proteins only in the lane containing MoAb MAd18-5D7 but not with the other tested IgM MoAbs or IgG MoAb. No reactivity was observed with any of the irrelevant phage clones or with wild-type phage wt pc89 (data not shown).

ELISA inhibition assay In an ELISA assay, phage displayed mimotopes 1 and 2 were tested for their ability to inhibit the binding of MAd18-5D7 antibody to recombinant Mel-CAM. Both mimotopes inhibited the binding of MAd18-5D7 to recombinant Mel-CAM in ELISA in a dose-dependent manner (**Fig 4*a***). An ELISA inhibition assay was performed with synthetic peptides to exclude that phage displayed peptides were constrained by the structural context of the bacteriophage surface. The result is shown in **Fig 4(*b*)** and demonstrates that inhibition occurs in a dose-dependent manner with the synthetic mimotopes 1 and 2 but not with the unrelated peptide ch3. Mimotope 1 inhibited 89% and mimotope 2 inhibited 54% of the binding of MAd18-5D7 to recombinant Mel-CAM. The unrelated peptide ch3 did not influence the binding characteristics of MAd-5D7. These data clearly show that the synthetic peptides possess an inhibitory capacity comparable to the phage displayed mimotopes.

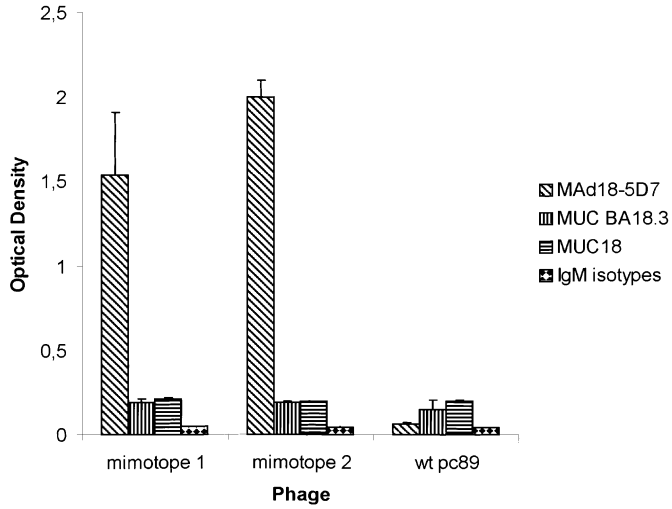

Figure 2. Phage ELISA to determine the reactivity of phage displayed mimotopes 1 and 2 and wild-type phage wt pc89 with the antibody MAd18-5D7. Microtiter 96-well plates were coated with anti-pIII antibody, and 5×10^{10} phages were added to each well, followed by MoAb MAd18-5D7, MUC BA18.3, MUC18, and an equimolar mix of two unrelated IgM antibodies (TEPC 183, MOPC 104E). Binding results are shown as A405 (mean and SD).

DISCUSSION

In melanoma cells and in more than 80% of metastatic lesions the cell surface adhesion molecule Mel-CAM is highly expressed (Kraus *et al*, 1997). The murine MoAb MAd18-5D7 directed against Mel-CAM is able to enhance Mel-CAM mediated adhesion of melanoma cells in aggregation assays (J. P. Johnson, Institute of Immunology, University of Munich, Munich, Germany, unpublished results). Our aim was to select low molecular weight structural equivalents (mimotopes) of the epitope of Mel-CAM that is recognized by MAd18-5D7 for future use as reagents to study the involvement of Mel-CAM in cell adhesion. Phage display peptide libraries are routinely used to characterize binding sites of antibodies and thereby define the epitope of the original antigen (Zwick *et al*, 1998; Moe *et al*, 1999). Commonly used phage libraries display peptides of up to 15 amino acid residues with random structure.

In this study we constructed and used for the first time a linear 28mer phage display peptide library to select mimotopes for the murine IgM MoAb MAd18-5D7. In the past several authors have already demonstrated that mimotopes can be obtained from peptide phage libraries and that these sequences can bind antibodies raised against native structures (Cwirla *et al*, 1990; Zwick *et al*, 1998). It has also been shown that mimotopes can mimic carbohydrate structures and oligonucleotide structures (Gaynor *et al*, 1997; Moe *et al*, 1999).

A random peptide library will contain a larger amount of diversity information for an increase in the length of the displayed peptide compared to shorter ones deriving from the same number of clones. At the same time the average number of copies of recombinant pVIII molecules displayed per phage particle will become smaller (Iannolo *et al*, 1995; Malik *et al*, 1996). The 28mer size of our random peptide library corresponds to the size of a small protein domain like that of a 26 amino acid Cys2His2 consensus "zinc-finger" motif used to produce a conformationally homogeneous combinatorial peptide library (Bianchi *et al*, 1995). We have no direct information on the structure of the selected 28mers.

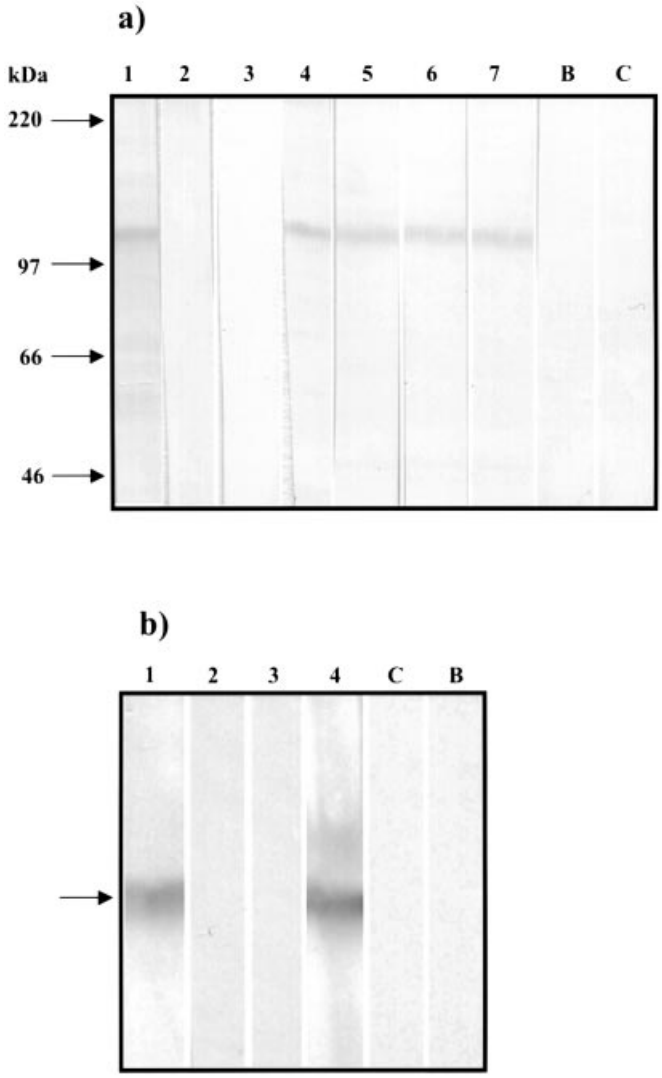

Figure 3. Immunoblot assay showing the ability of phage displayed mimotopes 1 and 2 to inhibit the binding of MoAb MAd18-5D7 to (*a*) natural and (*b*) recombinant Mel-CAM. (*a*) MelJuSo protein lysate or (*b*) 5 μg Mel-CAM *were* separated by 8% SDS-PAGE and proteins were transferred to a nitrocellulose membrane. Strips were incubated with MoAb MAd18-5D7 (*lane 1*), MoAb MAd18-5D7 preincubated with phage displayed mimotope 1 (*lane 2*), mimotope 2 (*lane 3*), or wild-type phage wt pc89 (*lane 4*), and for (*a*) with MoAb MUC BA18.3 (*lane 5*), and MoAb MUC BA18.3 preincubated with phage displayed mimotope 1 (*lane 6*) or mimotope 2 (*lane 7*). B, buffer control; C, isotype control; arrow in (*b*) indicates position of recombinant Mel-CAM.

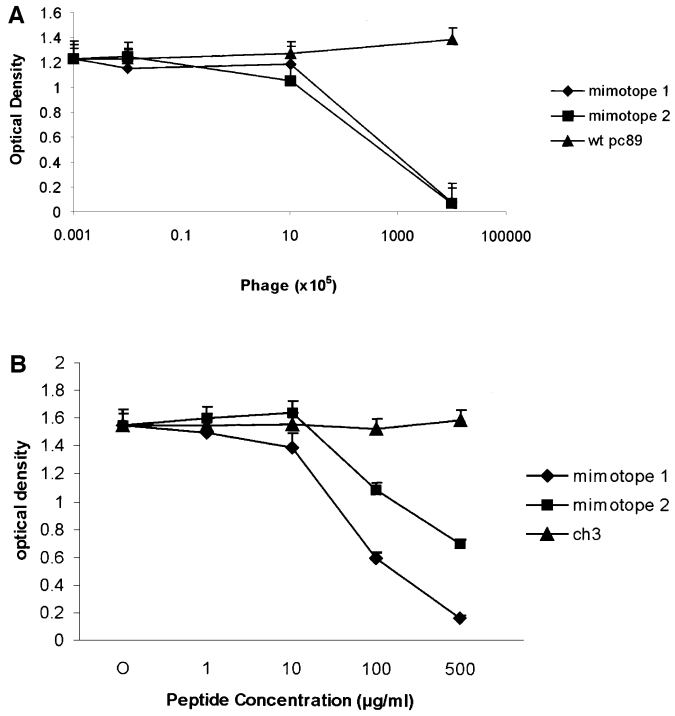

Figure 4. ELISA assays to determine peptide inhibition of MoAb MAd18-5D7 binding to recombinant Mel-CAM. (*a*) Inhibition of MoAb MAd18-5D7 binding to recombinant Mel-CAM by phage displayed mimotopes. MAd18-5D7 was preincubated with increasing concentrations of specific phage displayed peptides (mimotope 1 and 2) or wild-type phage wt pc89, and incubated with recombinant Mel-CAM coated to a microtiter plate. Binding of MAd18-5D7 to recombinant Mel-CAM was measured using an AP-conjugated antimouse IgM (μ-specific). Binding results are shown as A405 (mean and SD). (*b*) Synthetic peptide inhibition of MoAb MAd18-5D7 binding to recombinant Mel-CAM. MAd18-5D7 was preincubated with increasing concentrations of synthetic mimotopes 1 and 2 or unrelated synthetic peptide ch3, and incubated with recombinant Mel-CAM coated to a microtiter plate. Binding of MAd18-5D7 to recombinant Mel-CAM was measured using an AP-conjugated antimouse IgM (μ-specific). Binding results are shown as A405 (mean and SD).

In general, the role of residues flanking a conserved core sequence may be to provide minor, less specific interactions with the target or to constrain the conformation of the core motif (Katz, 1997).

We preincubated our 28mer phage display peptide library on purified mouse IgM to exclude peptides that bind to the constant region of an IgM molecule. The selection of ligand-binding phage particles was achieved by three rounds of panning of the library against the antibody MAd18-5D7 bound to a solid phase. The pool of phages from the third selection round was analyzed by sequencing of single phage clones. Two clones bearing peptide sequences that appeared more than once were selected for a more detailed study of their binding characteristics (**Fig 2**). Both clones that bound to MAd18-5D7 did not react with several IgM antibodies of various origins. This clearly demonstrated the absence of cross-reactivity of the selected phage clones. Furthermore, the binding of MAd18-5D7 to recombinant Mel-CAM could be inhibited by both phage displayed peptides (**Figs 3*b***, **4*a***). This inhibition was also shown with synthetic peptides to exclude that the conformations of the phage displayed peptides were constrained by the structural context of the bacteriophage surface (**Fig 4*b***). These results clearly indicate that the synthetic peptides adopted a conformation in solution that was comparable to the one on the phage surface. MoAb MUC BA18.3 also directed to Mel-CAM could not be inhibited by either of the two mimotopes (**Fig 3*a***). This proves that the mimotopes are genuine antigenic mimics of the MAd18-5D7 epitope on Mel-CAM.

By linear alignment using the BLAST algorithm it was possible to align the peptide sequence of the mimotope 1 to the sequence of Mel-CAM between amino acid positions 307–310 (LEPA) of the extracellular domain of Mel-CAM. These amino acid residues contribute to the third immunoglobulin-like domain of Mel-CAM. For mimotope 2 no linear alignment to the sequence of Mel-CAM could be found. This and the results of the inhibition studies illustrate that sequence homology between a mimotope and the original epitope is not obligatory for the formation of an equivalent structure.

Mimotopes can be used in many ways as is well documented in the literature. Phage clones isolated from peptide libraries are in

some cases able to induce an effectively specific humoral immune response *in vivo* directed against the epitope recognized by the MoAb used for affinity selection (Meola *et al*, 1995). Furthermore protective immune response by mimotope immunization has been shown for viral antigens, pollen allergens, and tumor antigens (Zucchelli *et al*, 2001; Jensen-Jarolim *et al*, 1998; Popkov *et al*, 2000).

The quantitative and qualitative changes between normal and malignant tumor cells are often shown in different expression of adhesion molecules. In malignant melanoma Mel-CAM expression gradually increases and is on the highest level in metastatic melanoma (Shih *et al*, 1994). Whereas human melanoma cells show a Mel-CAM-dependent adhesion to endothelial cells, it remains unclear if this requires Mel-CAM or Mel-CAM ligand expression (Shih *et al*, 1997). Genetic suppressor elements specific for Mel-CAM inhibit melanoma growth and invasion through loss of gap junction communication in a three-dimensional tissue reconstruct, suggesting that Mel-CAM affects connexin expression or acts *per se* as glue between cells and facilitates gap junction formation (Satyamoorthy *et al*, 2001). Another function that can be attributed to Mel-CAM is cation-independent homotypic adhesion of melanoma cells mediated through an unidentified ligand, which is not Mel-CAM (Johnson *et al*, 1997; Shih *et al*, 1997).

In summary, our data suggest that the described synthetic mimotopes can replace phage displayed mimotopes as the more useful tools for the careful study of the interaction of the MoAb MAd18-5D7 with melanoma cells. Ultimately, it may become possible to study the ability of the mimotopes to inhibit the interaction of a putative ligand with Mel-CAM, or the ability to interfere with the aggregation of melanoma cells. Such experiments should clarify whether the MAd18-5D7 epitope on Mel-CAM is involved in increased cell aggregation of melanoma cells and the formation of metastases.

We thank Dr. M. Herlyn (The Wistar Institute, Philadelphia, PA) for providing the recombinant Mel-CAM, and we thank Dr. J. P. Johnson (Institute of Immunology, University of Munich, Munich, Germany) for the Mel-CAM antibodies. This work was supported in part by the Medical Research Fund of the Mayor of Vienna, Project no. 1764, and by the Austrian National Bank Grant, Project no. 8572.

REFERENCES

Anfosso F, Bardin N, Frances V, Vivier E, Camoin-Jau L, Sampol J, Dignat-George F: Activation of human endothelial cells via S-endo-1 antigen (CD146) stimulates the tyrosine phosphorylation of focal adhesion kinase p125 (FAK). *J Biol Chem* 273:26852–26856, 1998

Bardin N, George F, Mutin M, *et al:* S-Endo 1, a pan-endothelial monoclonal antibody recognizing a novel human endothelial antigen. *Tissue Antigens* 48:531–539, 1996

Bianchi E, Folgori A, Wallace A, *et al:* A conformationally homogeneous combinatorial peptide library. *J Mol Biol* 247:154–160, 1995

Cwirla SE, Peters EA, Barrett RW, Dower WJ: Peptides on phage: a vast library of peptides for identifying ligands. *Proc Natl Acad Sci USA* 87:6378–6382, 1990

Dente L, Cesareni G, Micheli G, *et al:* Monoclonal antibodies that recognise filamentous phage: tools for phage display technology. *Gene* 148:7–13, 1994

Felici F, Castagnoli L, Musacchio A, Jappelli R, Cesareni G: Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector. *J Mol Biol* 222:301–310, 1991

Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B: Peptide inhibition of glomerular deposition of an anti-DNA antibody. *Proc Natl Acad Sci USA* 94:1955–1960, 1997

Holness CL, Simmons DL: Structural motifs for recognition and adhesion in members of the immunoglobulin superfamily. *J Cell Sci* 107:2065–2070, 1994

Iannolo G, Minenkova O, Petruzzelli R, Cesareni G: Modifying filamentous phage capsid: limits in the size of the major capsid protein. *J Mol Biol* 248:835–844, 1995

Jarolim E, Tejkl M, Rohac M, *et al:* Monoclonal antibodies against birch pollen allergens: characterization by immunoblotting and use for single-step affinity purification of the major allergen Bet v I. *Int Arch Allergy Appl Immunol* 90:54–60, 1989

Jensen-Jarolim E, Leitner A, Kalchhauser H, *et al:* Peptide mimotopes displayed by phage inhibit antibody binding to bet v 1, the major birch pollen allergen, and induce specific IgG response in mice. *Faseb J* 12:1635–1642, 1998

Johnson JP, Bar-Eli M, Jansen B, Markhof E: Melanoma progression-associated glycoprotein MUC18/MCAM mediates homotypic cell adhesion through interaction with a heterophilic ligand. *Int J Cancer* 73:769–774, 1997

Katz BA: Structural and mechanistic determinants of affinity and specificity of ligands discovered or engineered by phage display. *Annu Rev Biophys Biomol Struct* 26:27–45, 1997

Kraus A, Masat L, Johnson JP: Analysis of the expression of intercellular adhesion molecule-1 and MUC18 on benign and malignant melanocytic lesions using monoclonal antibodies directed against distinct epitopes and recognizing denatured, non-glycosylated antigen. *Melanoma Res* 7 (Suppl. 2):S75–S81, 1997

Lehmann JM, Holzmann B, Breitbart EW, Schmiegelow P, Riethmuller G, Johnson JP: Discrimination between benign and malignant cells of melanocytic lineage by two novel antigens, a glycoprotein with a molecular weight of 113,000 and a protein with a molecular weight of 76,000. *Cancer Res* 47:841–845, 1987

Lehmann JM, Riethmuller G, Johnson JP: MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. *Proc Natl Acad Sci USA* 86:9891–9895, 1989

Leitner A, Vogel M, Radauer C, *et al:* A mimotope defined by phage display inhibits IgE binding to the plant panallergen profilin. *Eur J Immunol* 28:2921–2927, 1998

Malik P, Terry TD, Gowda LR, *et al:* Role of capsid structure and membrane protein processing in determining the size and copy number of peptides displayed on the major coat protein of filamentous bacteriophage. *J Mol Biol* 260:9–21, 1996

Meola A, Delmastro P, Monaci P *et al:* Derivation of vaccines from mimotopes. Immunologic properties of human hepatitis B virus surface antigen mimotopes displayed on filamentous phage. *J Immunol* 154:3162–3172, 1995

Moe GR, Tan S, Granoff DM: Molecular mimetics of polysaccharide epitopes as vaccine candidates for prevention of Neisseria meningitidis serogroup B disease. *FEMS Immunol Med Microbiol* 26:209–226, 1999

Pickl WF, Majdic O, Fischer GF, *et al:* MUC18/MCAM (CD146), an activation antigen of human T lymphocytes. *J Immunol* 158:2107–2115, 1997

Popkov M, Sidrac-Ghali S, Alakhov V, Mandeville R: Epitope-specific antibody response to HT-1080 fibrosarcoma cells by mimotope immunization. *Clin Cancer Res* 6:3629–3635, 2000

Ruoslahti E: Targeting tumor vasculature with homing peptides from phage display. *Semin Cancer Biol* 10:435–442, 2000

Satyamoorthy K, Muyrers J, Meier F, Patel D, Herlyn M: Mel-CAM-specific genetic suppressor elements inhibit melanoma growth and invasion through loss of gap junctional communication. *Oncogene* 20:4676–4684, 2001

Shih IM, Elder DE, Speicher D, Johnson JP, Herlyn M: Isolation and functional characterization of the A32 melanoma-associated antigen. *Cancer Res* 54:2514–2520, 1994

Shih IM, Speicher D, Hsu MY, Levine E, Herlyn M: Melanoma cell–cell interactions are mediated through heterophilic Mel- CAM/ligand adhesion. *Cancer Res* 57:3835–3840, 1997

Shih IM: The role of CD146 (Mel-CAM) in biology and pathology. *J Pathol* 189:4–11, 1999

Smith GP: Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228:1315–1317, 1985

St Croix B, Rago C, Velculescu V, *et al:* Genes expressed in human tumor endothelium. *Science* 289:1197–1202, 2000

Versteeg R, Peltenburg LT, Plomp AC, Schrier PI: High expression of the c-myc oncogene renders melanoma cells prone to lysis by natural killer cells. *J Immunol* 143:4331–4337, 1989

Xie S, Luca M, Huang S, Gutman M, Reich R, Johnson JP, Bar-Eli M: Expression of MCAM/MUC18 by human melanoma cells leads to increased tumor growth and metastasis. *Cancer Res* 57:2295–2303, 1997

Zucchelli S, Roccasecca R, Meola A, *et al:* Mimotopes of the hepatitis C virus hypervariable region 1, but not the natural sequences, induce cross-reactive antibody response by genetic immunization. *Hepatology* 33:692–703, 2001

Zwick MB, Shen J, Scott JK: Phage-displayed peptide libraries. *Curr Opin Biotechnol* 9:427–436, 1998