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Monoclonal Antibodies Specific for the Empty Conformation of HLA-DR1 Reveal Aspects of the Conformational Change Associated with Peptide Binding*

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Class II major histocompatibility complex (MHC) proteins bind peptides and present them at the cell surface for interaction with $CD4^+$ T cells as part of the system by which the immune system surveys the body for signs of infection. Peptide binding is known to induce conformational changes in class II MHC proteins on the basis of a variety of hydrodynamic and spectroscopic approaches, but the changes have not been clearly localized within the overall class II MHC structure. To map the peptide-induced conformational change for HLA-DR1, a common human class II MHC variant, we generated a series of monoclonal antibodies recognizing the β subunit that are specific for the empty conformation. Each antibody reacted with the empty but not the peptide-loaded form, for both soluble recombinant protein and native protein expressed at the cell surface. Antibody binding epitopes were characterized using overlapping peptides and alanine scanning substitutions and were localized to two distinct regions of the protein. The pattern of key residues within the epitopes suggested that the two epitope regions undergo substantial conformational alteration during peptide binding. These results illuminate aspects of the structure of the empty forms and the nature of the peptide-induced conformational change.

Major histocompatibility complex (MHC)[1] molecules are heterodimeric cell-surface proteins that play an important role in the initiation of antigen-specific immune responses. Class II MHC proteins bind peptides derived from extracellular, endosomal, and internalized cell-surface antigens, and present them at the cell surface for inspection by $CD4^+$ T cells (1). Three-dimensional structures have been determined for peptide complexes of several polymorphic variants of both human and murine class II MHC molecules (reviewed in Ref. 2). Both the MHC α and β chains contribute to the peptide binding site, which is made up of a β sheet floor topped by two roughly parallel α helical regions. Each subunit contributes an immunoglobulin-like domain below the peptide binding site, as well as short transmembrane and cytoplasmic domains. Peptides bind in an extended conformation in the groove between the two helices, with ~10 residues able to interact with the MHC protein, and the peptide termini extending from the binding site. This conformation, similar to a polyproline type II helix, has a 2.7-residue repeat and appears to be dictated by a network of conserved hydrogen bonding interactions between the MHC and bound peptide (3). The conformation places 4–6 of the peptide side chains into pockets within the overall groove. The residues lining these pockets vary between allelic variants, providing different peptide-sequence binding specificity. Overall the interaction buries ~70% of the peptide surface area in the central region of a bound peptide, leaving the remainder available for interaction with antigen receptors on T cells (4).

Although the canonical structure visualized by x-ray crystallography is relatively stereotyped, a number of studies have suggested that alternate conformations of class II MHC molecules can exist under certain conditions (5–8). Kinetic studies of the peptide-binding reaction indicate a multistep binding pathway, suggesting that peptide binding is accompanied by a conformational change in the MHC, although other interpretations of these kinetic analyses are possible (9–14). Conformational transitions between such isomers have been measured by NMR (15). Other studies have shown that at low pH, MHC molecules shift their equilibrium from a "closed" to an "open" or "peptide-receptive" state (16–18). These alternate conformations have been suggested to play an important role during the peptide-binding reaction, and also in the interaction with the endosomal peptide exchange factor HLA-DM (6, 19–21).

Little structural information is available for the empty form of the protein. Spectroscopic and hydrodynamic studies on HLA-DR1, a common human class II protein and the subject of this study, have shown that a distinct conformational change occurs upon peptide binding, suggesting that the conformation of the empty protein is different from that of the peptide-loaded

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[1] The abbreviations used are: MHC, major histocompatibility complex; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; BSA, bovine serum albumin; ABTS, 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid); SPR, surface plasmon resonance.

form (8, 22). The conformational change results in a decrease in hydrodynamic radius from 35 to 29 Å, together with a small increase in helical content as observed by circular dichroism (8). The change can be induced by binding any of a large variety of peptides, including a capped dipeptide (5), and also by filling the P1 side chain binding pocket through mutagenesis (7).

Conformation-sensitive monoclonal antibodies have long been used to investigate structural properties of proteins (23). These antibodies are able to distinguish between two or more structural forms of a protein, and are useful as structural and biological probes of the molecular surface. Mapping the epitopes of such antibodies can provide information on the nature of the structural change and on the location of the regions involved (24). In previous work, the happenstance cross-reactivity of an antibody raised against the murine MHC protein I-A^s was used to help map regions involved in the peptide-induced conformational change in human HLA-DR1, which is only 66% identical with I-A^s. In the present work, a set of monoclonal antibodies directed against the β chain of HLA-DR1, raised by immunization with unfolded β subunit, was screened for the ability to distinguish between empty and peptide-loaded conformations of the intact, folded protein. In an effort to gain site-specific structural information about the peptide-induced conformational change, the epitope of each of these antibodies was mapped. Two distinct regions of the MHC β chain that are accessible in the empty but not peptide-loaded conformation were identified: one nearby but not coincident with the peptide binding site, and one across the molecule near the membrane-spanning region.

EXPERIMENTAL PROCEDURES

Recombinant MHC Molecules

Empty and peptide-loaded DR1 were prepared by expression in *Escherichia coli* and folded *in vitro*, as previously described (25). Briefly, HLA-DR1 extracellular domains were expressed individually as insoluble inclusion bodies, isolated by denaturing ion exchange chromatography, and refolded *in vitro* without peptide. Peptide complexes were prepared by incubating immunoaffinity purified empty HLA-DR1 (1–5 μM) with at least 5-fold molar excess peptide for 3 days at 37 °C in PBS. The resultant peptide-DR1 complexes (or HLA-DR1 that had not been loaded with peptide and had been stored at 4 °C) were purified by gel filtration to remove aggregates and unbound peptide, and stored at 4 °C. HLA-DR allelic variants other than HLA-DR1 (and HLA-DRB5*0101 (26)) have been difficult to prepare by this method, and so HLA-DR4 and HLA-DR52 (and control HLA-DR1) were produced instead using an insect cell expression system (27, 28). Briefly, HLA-DR extracellular domains were secreted by *Drosophila* S2 cells transfected with genes carrying endogenous signal sequences, and HLA-DR proteins were isolated from conditioned medium by immunoaffinity and loaded with peptide as described above for HLA-DR1 from *E. coli*.

Peptide Synthesis and Purification

Peptides were synthesized using an Advanced ChemTech 490 synthesizer and standard Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) chemistry. Biotinylated peptides were produced by addition of biotin-LC-LC-NHS ester (succinimdyl-6′-(biotinamido)-6-hexanamido hexanoate; Pierce) to the deprotected NH_2 terminus of each peptide while still on the solid support. The reaction was carried out overnight in dimethylformamide with a catalytic amount of diisopropylethylamine. Crude peptides carrying either free amino- or biotin-LC-LC termini were deprotected and cleaved with a solution of 83% trifluoroacetic acid, 5% phenol, 5% water, 5% dithiothreitol, and 2% triisopropylsilane, and then precipitated in ether, washed, and lyophilized. Peptides were purified by reverse phase high performance liquid chromatography using a gradient of 2–100% acetonitrile over 80 min. Purity was determined by analytical high performance liquid chromatography and mass spectrometry.

Development of Monoclonal Antibodies

Mice (BALB/c x B10.A F1 hybrid) were immunized with purified, insoluble DR1 β chain (DRB1*0101) expressed in *E. coli* inclusion bodies. Hybridomas were obtained by standard techniques, including fusion with Sp2/0 myeloma, selection in hypoxanthine/aminopterin/thymidine medium, and repeated cloning by limiting dilution. After primary ELISA screening of hybridoma supernatants for reactivity with the denatured DR1 β subunit immunogen, the positive supernatants were screened for their ability to distinguish empty DR1 from HA-peptide-loaded DR1 using enzyme-linked immunosorbent assay (ELISA) (see below). After three rounds of subcloning and screening, four monoclonal hybridomas were selected for further study. Each of the four antibodies selected (MEM-264, MEM-265, MEM-266, and MEM-267) reacted with empty but not HA-peptide-loaded DR1 as well as several other DR allelic variants (see below). For Western blotting, purified DR proteins, or whole cell lysates of B cells or transfectants, were separated by SDS-PAGE, transferred to nitrocellulose or Immobilon-P (Millipore), and blocked with 1% BSA before incubation with the monoclonal antibodies. Bound antibodies were detected using alkaline phosphatase-coupled, affinity purified, goat anti-mouse IgG. For epitope mapping and cellular studies, the monoclonal antibodies were produced either as ascites in mice or in hybridoma culture using serum-free medium (Hybridoma SFM, Invitrogen), and were purified by protein A affinity chromatography. The antibody class of each monoclonal antibody was determined by enzyme immunoassay using a mouse-hybridoma subtyping kit (Roche).

Sandwich ELISA

A sandwich ELISA was used for determination of the relative binding of antibodies to empty and peptide-loaded HLA-DR1. Purified antibodies (1 μg/ml unless noted) were immobilized onto Dynatech Immobilon-4 polystryrene 96-well plates, by incubation for 4 h at room temperature (or overnight at 4 °C). Nonspecific binding sites were blocked by incubation with 3% BSA in PBS overnight at 4 °C. Antibodies present in ascites fluid or hybridoma culture supernatant were bound to goat anti-mouse IgG that had been immobilized as described above for purified antibodies. After antibody coating, the plates were washed three times with TBST (25 mM Tris, 0.15 M NaCl 0.05% Tween 20, pH 7.4). Dilutions of empty HLA-DR1, HA-peptide-loaded HLA-DR1, or HLA-DR1 complexes with other peptides were prepared in PBS containing 0.1% Tween 20 and 0.3% BSA, and incubated with the immobilized antibodies for 2 h at 25 °C. After binding, the plates were washed three times with TBST, incubated for 1 h at 25 °C with rabbit anti-DR1 antiserum ("CHAMP") diluted 1/25,000 in PBST, and washed again. Finally, the plates were incubated with peroxidase-labeled goat anti-rabbit Fc (Roche) diluted 1/4,000, washed, and developed using the colorimetric peroxidase substrate ABTS (Roche). Absorbance increase because of peroxidase activity (405 nm) was measured in a microtiter plate reader (Wallac, PerkinElmer Life Sciences). For complexes of the very weakly bound peptide YRAL, excess peptide was included in the HLA-DR1 incubation. Apparent antibody binding affinities and optimum pH for each antibody was determined using this ELISA as well.

A variation of the sandwich ELISA was used to estimate antibody-peptide affinity. Plates were coated with MEM antibodies, blocked, and washed as above, and then incubated with varying concentrations of biotinylated peptide for 1 h at 25 °C. Plates were washed three times with TBST, and binding was detected with peroxidase-labeled streptavidin and the colorimetric peroxidase substrate ABTS, as above. The concentration of half-maximal binding is reported as a measure of relative binding affinity.

Another variation of the sandwich ELISA was used to confirm that the antibodies react with intact DR1 $\alpha\beta$ heterodimers and not only isolated β subunits. Plates were coated with MEM antibodies, blocked, and washed as above, and then incubated for 1 h at 25 °C with various concentrations of empty DR1 that had been biotinylated on the cysteine residue introduced at the COOH terminus of the α chain (29). Plates were washed three times with TBST, and binding was detected with alkaline phosphatase-labeled streptavidin and the colorimetric substrate *p*-nitrophenyl phosphate (both from KPL, Gaithersburg, MD).

Epitope Mapping

Direct Binding ELISA—For epitope mapping, antibody-peptide interaction was measured using a direct ELISA with immobilized peptides and immunochemical detection of bound antibody. Ninety-six-well microtiter plates (as above) were coated with streptavidin (Prozyme, San Leandro, CA) at a concentration of 500 ng/well (overnight at 4 °C, in PBS). Plates were then blocked with 3% BSA in PBS for 3 h at 25 °C to block nonspecific binding sites. After washing three times with TBST, biotinylated peptides were added at a concentration of 10 μM in PBS containing 0.1% Tween 20 and 0.3% BSA and allowed to bind for 1 h at 25 °C. Plates were washed with TBST and test monoclonal

antibodies (100 μl of 1 μg/ml monoclonal antibody) were added, followed by incubation for 1 h at 25 °C. Plates were again washed with TBST three times and binding was determined using a peroxidase-labeled goat anti-mouse antibody, as above (Roche). Plates were developed using ABTS and absorbance was measured in a microtiter plate reader.

Competition ELISA for Peptide Screening—Antibody-peptide interaction was also measured using a competition ELISA, in which soluble peptide and empty HLA-DR1 competed for binding to immobilized antibody, with immunochemical detection of bound HLA-DR1. Microtiter plates were coated with test monoclonal antibodies (100 μl at a concentration of 1 μg/ml) overnight at 4 °C. Plates were then blocked and washed as above and incubated with or without peptides. After a 30-min peptide incubation, 100 ng of empty HLA-DR1 was added to each well (peptides were not washed away). DR1 binding was measured as above, using rabbit anti-DR1 antiserum (1/25,000) followed by peroxidase-labeled goat anti-rabbit Fc (1/4,000) as the secondary and detection antibodies, respectively. Plates were developed using ABTS and absorbance was measured in a microtiter plate reader.

Surface Plasmon Resonance (SPR) SpotMatrix Analysis—SpotMatrix SPR analysis was performed using an Applied Biosystems 8500 Affinity Chip Analyzer, with immobilized peptides and surface plasmon resonance detection of bound antibody. Biotinylated peptides were spotted using a SMP10B pin (Telechem) on a MicroSys spotter (Cartesian Dispensing Systems, presently Genomic Solutions) at a concentration of 25 μg/ml (~10 mM) in 20 μl of either Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal calf serum or 4 mg/ml BSA in PBS. Each spot had an approximate diameter of 330–360 μm and a volume of 2.5 nl per spot. Multiple copies of each chip were made and tested. Following chip equilibration for approximately 1 h in PBST at a flow rate of 0.5 ml/min, 5 ml of test monoclonal antibody at the indicated concentration in PBST (10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, 0.05% Tween, pH 7.4) was flowed over the SpotMatrix at a flow rate of 0.5 ml/min for 8 min. Dissociation was monitored over a 10-min time span. End point binding was calculated after background subtraction of individual reference spots from the generated SpotMatrix affinity traces (RCU, resonance change units, as a function of time). One RCU corresponds to a 1-millidegree SPR shift. The end point consisted of the averaged RCU values for the last minute of the association/equilibrium phase prior to the start of dissociation.

Analysis of Cell Surface MHC on Immature Dendritic Cells

Cell surface expression of empty and total HLA-DR1 on immature dendritic cells and control splenocytes was assessed by flow cytometry. Bone marrow-derived dendritic cells were prepared as previously described by culture of bone marrow cells from class II-deficient mice transgenic for a chimeric DRB1*0401 molecule (a gift of B. Huber) or from normal non-transgenic C57BL/6 mice. Bone marrow cells depleted for red blood cells were cultured (1–2 × 10^6 cells/ml) in the presence of granulocyte-macrophage colony-stimulating factor and interleukin 4 (10 ng/ml each, R&D Systems) in RPMI medium supplemented with 10% fetal bovine serum, HEPES (10 mM), glutamine, and penicillin/streptomycin (100 units each/ml). The cultures were replenished with cytokines every third day before harvesting for staining with MEM antibodies. Cells were harvested carefully by using cell lifters (COSTAR) to avoid unwanted cell damage/injury, washed in FACS buffer (PBS containing 1% BSA, 0.1% sodium azide, 1 mM EDTA, pH 7.2), and suspended in the same buffer. Approximately 1 × 10^5 cells were stained with saturating amounts of antibody for 45 min on ice, washed three times with FACS buffer, and incubated with fluorescein-conjugated $(Fab')_2$ anti-mouse IgG (H+L) secondary antibody (Jackson ImmunoResearch) for 45 min. After incubation, the cells were washed three times, suspended in 300 μl of buffer, and analyzed immediately using a FACScalibur flow cytometer (BD Biosciences). Propidium iodide (BD Pharmingen) negative cells were gated for analysis. Splenocytes used as a control were obtained from the same mice and were used freshly after red blood cell lysis.

RESULTS

Generation of Conformation-specific Monoclonal Antibodies—Both class I and class II MHC proteins undergo conformational changes upon their binding peptide ligands, as observed by spectroscopic, hydrodynamic, and immunological criteria (30–34). The human class II MHC protein HLA-DR1 can be obtained in well characterized empty and peptide-loaded forms by separately expressing the α and β subunits in *E. coli* and subsequently refolding the subunits together *in vitro* in the absence or presence of peptide ligand (25). Previously, we have used these species to characterize the peptide-induced conformational change in HLA-DR1 (5, 8, 35). Binding of any of a variety of peptide ligands induces a distinct conformational change, characterized by a decrease in hydrodynamic radius, an increase in per-residue molar ellipticity, and changes in binding of an antibody recognizing a polymorphic epitope on the β subunit. Similar changes have been shown for mouse homologues of HLA-DR1 (32, 34). To probe in more detail the structural changes involved in the ligand-induced conformational change in HLA-DR1, a panel of murine monoclonal antibodies that distinguish the empty and peptide-loaded conformations were generated. Antibodies were raised by immunization with purified, denatured β subunit, and hybridomas were screened using a sandwich ELISA for their ability to preferentially bind to the empty but not the peptide-loaded form of HLA-DR1 (see "Experimental Procedures" for details). Four antibodies (MEM-264, MEM-265, MEM-266, and MEM-267) were selected based upon these characteristics. Each of the antibodies specifically binds to the empty but not peptide-loaded form of HLA-DR1 (Fig. 1*A*). Another antibody, LB3.1 (36, 37), does not distinguish the two forms of the protein, and binds both forms (Fig. 1*B*). Although the MEM epitopes are present on the β subunit, all MEM antibodies capture DR αβ heterodimers as efficiently as the αβ complex-specific antibody LB3.1 (Fig. 1*C*).

Each of the antibodies was tested using a variety of different peptide complexes, to determine whether they were sensitive to the peptide-induced conformational change regardless of the peptide sequence, and to rule out the possibility that antibody binding might simply be blocked by particular features of the HA peptide used in the initial screen. In each case, the MEM antibody bound only to the empty complex, and not to any of the MHC-peptide complexes tested (Fig. 2). The set of peptides tested had completely different sequences with different MHC-interacting anchor residues (*bold* in Fig. 2*A*), and included both high-affinity viral antigens and self-peptides, as well as designed variants of lower binding affinity and decreased half-life (5, 38). The MHC-peptide complex with CLIP, a fragment of the class II-associated invariant chain chaperone, and an important intermediate in the class II biosynthetic pathway, was not recognized by the MEM antibodies. Importantly, even the minimal binding tetra-peptide YRAL, which occupies only the NH_2-terminal half of the peptide binding site (5), is able to completely abrogate the binding of the MEM antibodies. Thus, each of the MEM antibodies distinguishes empty and peptide-loaded forms of HLA-DR1, without regard for the detailed nature of the bound peptide.

The reactivity of the MEM antibodies was characterized in more detail. Antibodies MEM-264, MEM-265, and MEM-267 are of the IgG2b isotype, whereas MEM-266 is IgG2a, and all carry the κ light chain. Each of the antibodies reacted strongly by Western blotting with reduced and denatured DR1 β subunits in whole cell lysates of B cells and transfectants expressing DR1, but not with SDS-solubilized (not boiled) samples from the same cells that contain native DR1αβ-peptide complexes (4). Each antibody reacted also with an unidentified band of ~80 kDa present in lysates of a variety of cells, and MEM-266 also reacted with several other unidentified bands. Other class II MHC proteins, HLA-DQ1 and DP4, were not recognized in whole cell lysates of transfected cells by any of the antibodies. The sandwich ELISA was used to determine the optimum binding pH and half-maximal binding affinity for each antibody (Table I). MEM-264, MEM-265, and MEM-267 had pH optima between 5 and 6, whereas MEM-266 bound maximally at pH 7. At the appropriate pH, all four of the

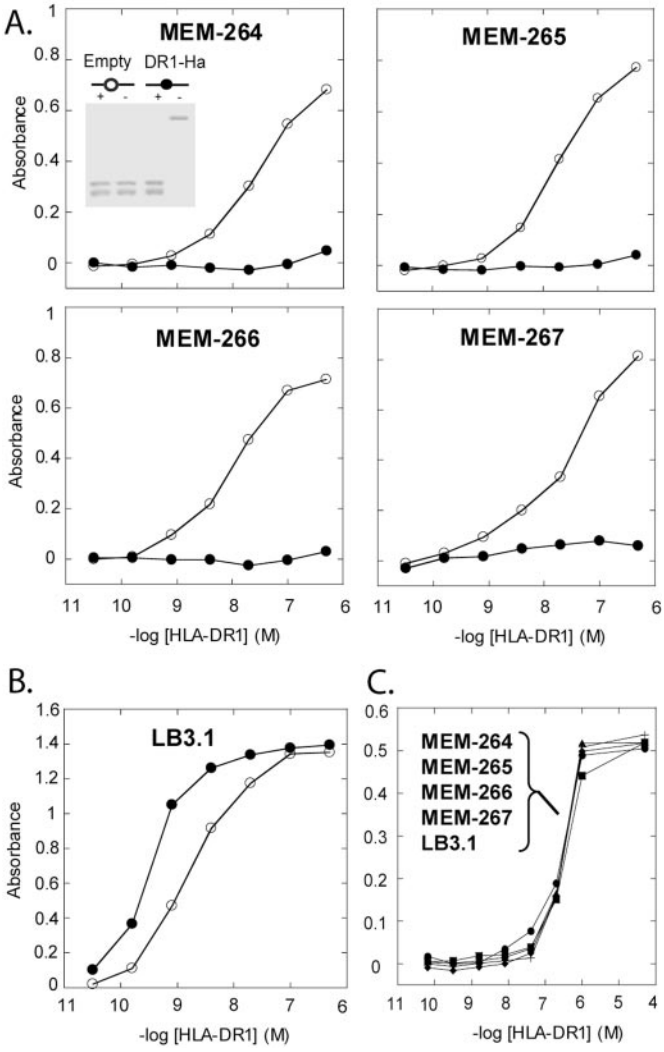


FIG. 1. **MEM antibodies specifically recognize empty conformation of DR1.** *A*, sandwich ELISA using immobilized conformation-specific MEM antibodies MEM-264, MEM-265, MEM-266, and MEM-267 and rabbit anti-DR antiserum detection. Each MEM antibody binds empty DR1 (*open circles*) and does not bind DR1-Ha (*closed circles*). *B*, same as *panel A* except using the anti-DR1 monoclonal antibody LB3.1, which recognizes both empty DR1 and DR-Ha. *C*, MEM antibodies recognize the DR1 $\alpha\beta$ heterodimers. Sandwich ELISA use immobilized MEM antibodies (or LB3.1) for capture of DR1 carrying a COOH-terminal biotin label on the α chain, with streptavidin detection. (LB3.1, *circles*; MEM-264, *squares*; MEM-265, *diamonds*; MEM-266, *triangles*; MEM-267, *plus*). DR α chains are captured as efficiently by the MEM antibodies as by the $\alpha\beta$ heterodimer-specific antibody LB3.1 (37). *Inset* to *panel A*, *upper left*, SDS-PAGE analysis of empty DR1 and DR1-Ha with samples in alternating lanes boiled (+) or kept at room temperature (not boiled, −) before loading. DR1-Ha (but not empty DR1) is resistant to SDS-induced subunit dissociation, indicative of peptide loading (62).

antibodies bound tightly to the empty form with apparent binding affinities below 100 nM.

HLA-DR Allele Specificity of MEM Binding—The binding of the MEM antibodies to allelic variants of HLA-DR1 was analyzed to evaluate their sequence specificity, to provide initial epitope mapping information, and to evaluate their potential application to biological problems involving various alleles. Genes coding for the β subunit of HLA-DR proteins are among the most polymorphic genes characterized in the human population, with over 400 alleles identified that differ by 1 to ~20 residues between protein variants (39). (The α subunit of HLA-DR1 is conserved, with only 2 alleles currently identified characterized by a single amino acid polymorphism.) Of this large

A.

Peptide	Sequence	Source	Kd, nM
HA	PK**Y**VK**Q**N**T**LK**L**AT	viral	14
CLIP	K**M**RM**A**T**P**LL**M**QALPM	endogenous	25
A2	VGSD**W**RFL**R**GYH**Q**YA	endogenous	17
HIV	PE**V**IP**M**F**S**AL**S**EGATP	viral	86
HAL	PK**Y**VK**Q**N**T**L	designed	N.D.
YRAL	Ac-**Y**RA**L**-NH2	designed	16,000

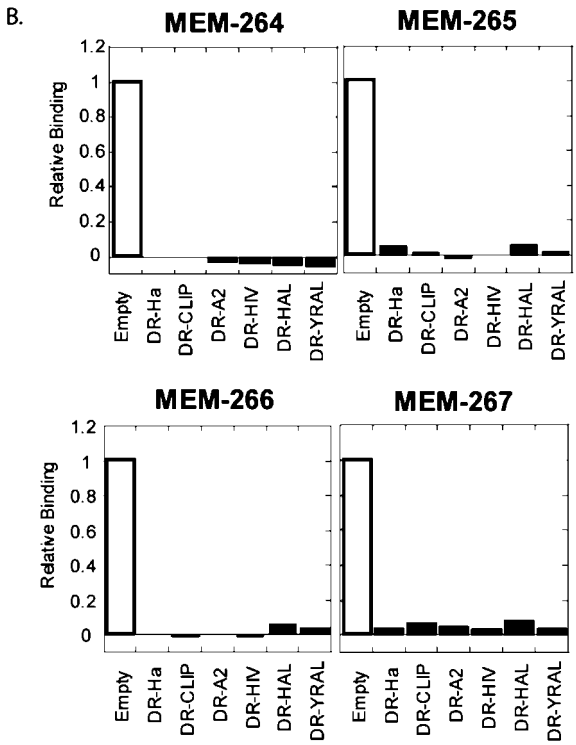


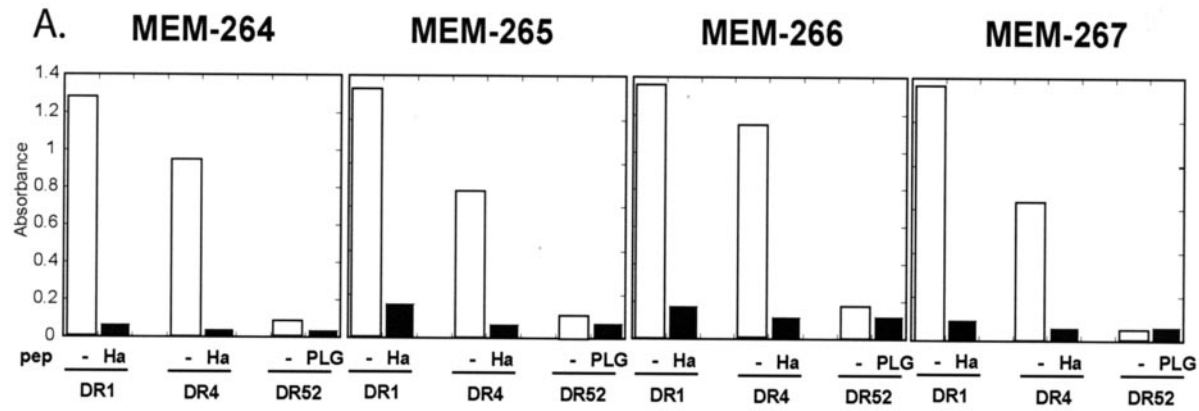
FIG. 2. **MEM antibody recognition of empty but not peptide-loaded DR1 does not depend on the nature of the bound peptide.** *A*, peptide sequences; *B*, MEM binding to DR1-peptide complexes as determined by sandwich ELISA as described in the legend to Fig. 1. Excess peptide was included during the MHC-peptide incubation step for the very weakly binding peptide Ac-YRAL-NH_2.

TABLE I
Antibody characterization

Protein binding and pH optima were determined by sandwich ELISA using immobilized MEM antibody, soluble DR1, and rabbit polyclonal anti-DR1 antibodies, with anti-rabbit detection. Binding to peptides was determined by ELISA using immobilized MEM antibodies, biotinylated peptide (bio-GGVTELGRPDAEYWNSQKDL for MEM-264, −265, and −267, and bio-GGSPLTVEWRA for MEM-266) followed by streptavidin detection.

Antibody	Subtype	pH optimum	Half-maximal binding	
			Protein	Peptide
			nM	
MEM-264	IgG2b	5-6	31	13400
MEM-265	IgG2b	5-6	19	7400
MEM-266	IgG2a	7	10	1
MEM-267	IgG2b	5-6	50	6450

set of polymorphic genes, only two have been expressed as recombinant empty proteins in *E. coli*, HLA-DRB1*0101 (coding for the β subunit of HLA-DR1) (25) and the closely related HLA-DRB5*0101 (coding for the β subunit of HLA-DR2a) (26). To investigate the specificity of the MEM antibodies to less closely related variants, insect cells were used as an alternative expression system. Several mouse and human class II MHC proteins have been expressed in insect cells (4, 40, 41). Peptide-free preparations can be obtained for many class II MHC pro-



B.

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                    10         20         30         40         50         60
DRB1*0101 : GDTRPRFLWQLKFECHFFNGTERVRLLERCIYNQEESVRFDSDVGEYRAVTELGRPDAEY : DR1
DRB1*0401 : --------E-V-H------------F-D-YF-H---Y---------------------- : DR4
DRB3*0101 : --------ELR-S------------Y-D-YFH----FL------------------V--S : DR52a

                    70         80         90        100        110        120
DRB1*0101 : WNSQKDLLEQRRAAVDTYCRHNYGVGESFTVQRRVEPKVTVYPSKTQPLQHHNLLVCSVS : DR1
DRB1*0401 : ----------K------------------------Y-E-----A--------------N : DR4
DRB3*0101 : ----------K-GR--N------------------H-Q-----A---------------- : DR52a

                   130        140        150        160        170        180
DRB1*0101 : GFYPGSIEVRWFRNGQEEKAGVVSTGLIQNGDWTFQTLVMLETVPRSGEVYTCQVEHPSV : DR1
DRB1*0401 : -------------------T-------------------------------------L : DR4
DRB3*0101 : ------------------------------------------------------------ : DR52a

                   190
DRB1*0101 : TSPLTVEWRA : DR1
DRB1*0401 : ---------- : DR4
DRB3*0101 : --A-----S- : DR52a
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FIG. 3. **Allele specificity of MEM antibodies.** *A*, all of the MEM antibodies bind to empty HLA-DR1 and DR4, but not to their peptide-loaded forms. None bind to DR52a in either the empty or peptide-loaded forms. *Open bars* correspond to peptide-free, empty forms; *closed bars* correspond to peptide-loaded forms. Assay by sandwich ELISA was as described in the legend to Fig. 1. *B*, sequences of the β subunits of DR1, DR4.1, and DR52a proteins, shown with corresponding gene names, and identity with DR1 indicated by *dashes*.

teins with careful control of culture conditions, although some alleles are unstable in the absence of peptide (42–45), and sometimes the MHC proteins co-purify with mixtures of adventitiously bound endogenous insect or medium-derived peptides (41, 45). Peptide-free preparations of HLA-DR1, HLA-DR4 (carrying the protein product of the HLA-DRB1*0401 gene), and HLA-DR52a (DRB3*0101 gene) were obtained using insect cell expression systems, and the corresponding peptide-loaded forms by *in vitro* loading with appropriate peptides (HA for DR4, and PLG, an integrin variant for DR52a (46)). These proteins were tested for their ability to bind to the MEM antibodies using the sandwich ELISA (Fig. 3*A*). None of the peptide-loaded forms bound to any of the antibodies. MEM-264, MEM-265, MEM-266, and MEM-267 all bound to the empty forms of DR1 and DR4, but none bound to the empty form of DR52a. Thus, the binding epitopes for the MEM antibodies are likely to include at least some of the 11 positions distributed throughout the sequences where DR1 and DR4 are identical but different than DR52a (Fig. 3*B*). A more comprehensive mapping study was undertaken to better define the location of these epitopes (see below).

Cellular Binding—Previously, empty or peptide-receptive forms of the mouse class II MHC proteins I-A^s, I-A^k, and I-A^u have been observed on the surface of immature dendritic cells (47). Most other murine cells that express class II MHC proteins, including B cells and macrophages, generally display only peptide complexes at their surface. Whether human MHC proteins behave similarly is not known. An important potential application of the MEM antibodies would be the detection and analysis of empty human HLA-DR molecules at the cell surface. To determine whether HLA-DR proteins could be expressed as empty molecules at the cell surface in a form recognized by the MEM antibodies, dendritic cells were prepared from HLA-DR4 transgenic mice, using a conventional *in vitro* differentiation procedure in which bone marrow-derived precursors develop into immature dendritic cells during culture in granulocyte-macrophage colony-stimulating factor (48). Immature dendritic cells from $DR4^+$ transgenic mice exhibited substantial cell surface reactivity with the anti-DR antibody LB3.1, and also with each of the MEM antibodies (Fig. 4). Splenocytes from these mice did not bind the MEM antibodies, although LB3.1 still bound (Fig. 4) showing that the lack of MEM antibody staining was not because of a lack of DR4 expression in these cells. None of the antibodies stained control

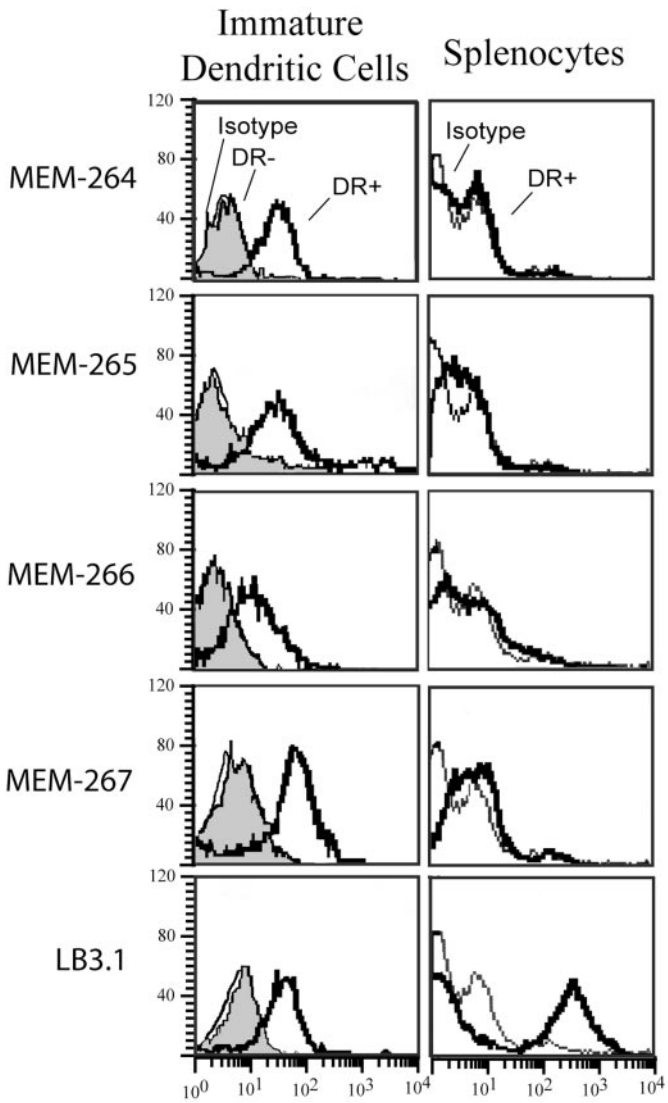

FIG. 4. **The MEM epitope can be detected at the cell surface.** MEM antibodies stained immature dendritic cells (*left*) that express empty cell surface MHC molecules (47), but not splenocytes (*right*) that express predominantly peptide loaded forms, whereas LB3.1 stained both cell types. *Thick lines*, antibody staining of cells from DR4+ transgenic mice; *thin lines*, isotype control. None of the antibodies stained control non-transgenic DR4-negative C57BL/6 immature dendritic cells (*shaded*).

cells that lacked the DR4 transgene. Immature dendritic cells from DR1-transgenic mice showed similar behavior (not shown). These results indicate that HLA-DR4, like I-A^s, can be expressed as an empty molecule in immature dendritic cells, and that the cell type-specific regulation of antigen processing pathways is similar for the DR transgenes as for endogenous mouse I-A proteins.

Peptide Mapping the MEM Epitopes—The MEM epitopes were mapped using a series of peptides 20 amino acids in length, each overlapping by 10 residues, and spanning the entire sequence of the HLA-DR1 β chain (Fig. 5A). This peptide mapping strategy (49) assumes that each antibody epitope is contained within a short, contiguous stretch of polypeptide, and has been used successfully to map many antibody epitopes (50–52). Here, the antibodies had been raised using denatured DR1 β subunit as an immunogen, which we did not expect to elicit antibodies recognizing conformational epitopes or epitopes including non-localized groups of residues. The series of overlapping peptides was tested initially in a direct binding immunoassay, using biotinylated peptides immobilized on streptavidin-coated wells (Fig. 5*B*). For each antibody, a single peptide gave a clear binding signal. MEM-264, MEM-265, and MEM-267 each bound to DRβ-(50–69), whereas MEM-266 bound to the C-terminal peptide DRβ-(170–190). To confirm these results and to establish that the peptides bound to the antigen-combining site of the antibody and not adventitiously at another location on the antibody, the series of overlapping peptides was retested using a competition assay, in which peptide and empty DR1 compete for binding to immobilized antibody (Fig. 5*C*). The same mapping results were obtained. Finally, antibody binding to the series of peptides was evaluated using a new SpotMatrix-based SPR technology. With this technology, binding of fluid-phase protein to a set of immobilized ligands is detected in parallel by surface plasmon resonance. Biotinylated peptides were immobilized by spotting them onto a streptavidin-coated gold chip, and monitored binding for the MEM antibodies in PBS solution. End point binding values for each peptide (Fig. 5*D*) reflect the same binding specificity as observed in the direct binding and competition assays. The SpotMatrix SPR technology provides information on both association and dissociation phases of the binding interaction, in addition to the end point values. A comparison of apparent dissociation constants measured for each epitope and alanine scan peptides will be discussed elsewhere.[2]

Fine Mapping of Monoclonal Antibody MEM-266—The MEM-266 epitope was defined more precisely using a submapping strategy. A series of 10-mer peptides overlapping by 7 residues, spanning the binding 20-mer peptide DRβ-(170–190), was tested, because typical linear epitopes are 5–7 amino acids in length (49, 53). Only the most COOH-terminal peptide BRβ-(182–190) bound (Fig. 6*A*). An alanine scan of this peptide revealed a linear epitope with predominant contributions from Trp-188 and Arg-189 and a smaller contribution from Val-186 (Fig. 6, *B* and *C*). This epitope explains the observed allele specificity of MEM-266 (Fig. 3*B*), as DR52a contains a substitution at position 189 (Arg to Ser).

Fine Mapping of MEM-264, MEM-265, and MEM-267 Antibodies—As an aid to the fine mapping studies of the MEM-264, -265, and -267 epitopes, binding peptides shorter than the 20-mer DRβ-(50–69) peptide originally observed in the primary screen were sought. A submapping strategy similar to that described for MEM-266 was unsuccessful, as none of the antibodies bound to a series of four overlapping 10-mers spanning the DRβ-(50–69) sequence (not shown). We attempted to identify a minimal peptide by truncation analysis. Only 2–3 residues could be removed from either the NH_2- or COOH-terminal end without abrogating binding (Fig. 7*A*). The resultant peptides are much longer than typically found for linear epitopes (49, 53) and if found in an extended conformation would be >50 Å, much longer than a typical antibody-combining site (~15–20 Å) (54). Only minor differences were observed between the binding behavior of MEM-264, MEM-265, and MEM-267 toward the truncation series. The 18-mer DRβ-(50–67) bound well to all of the antibodies and was selected for further mapping by alanine scanning. MEM-264, MEM-265, and MEM-267 each exhibited essentially the same pattern of sensitivity to alanine substitution, with important residues identified as Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67 (Fig. 7*B*). These residues are arranged in an approximate 3.5-residue repeat, suggestive of a helical or other ordered structure in the peptide conformation recognized by the antibody. The epitope explains the reactivity of MEM-264, MEM-

[2] V. Horejsi, R. F. Baggio, G. J. Carven, M. Palmer, L. J. Stern, J. E. Arenas, manuscript in preparation.

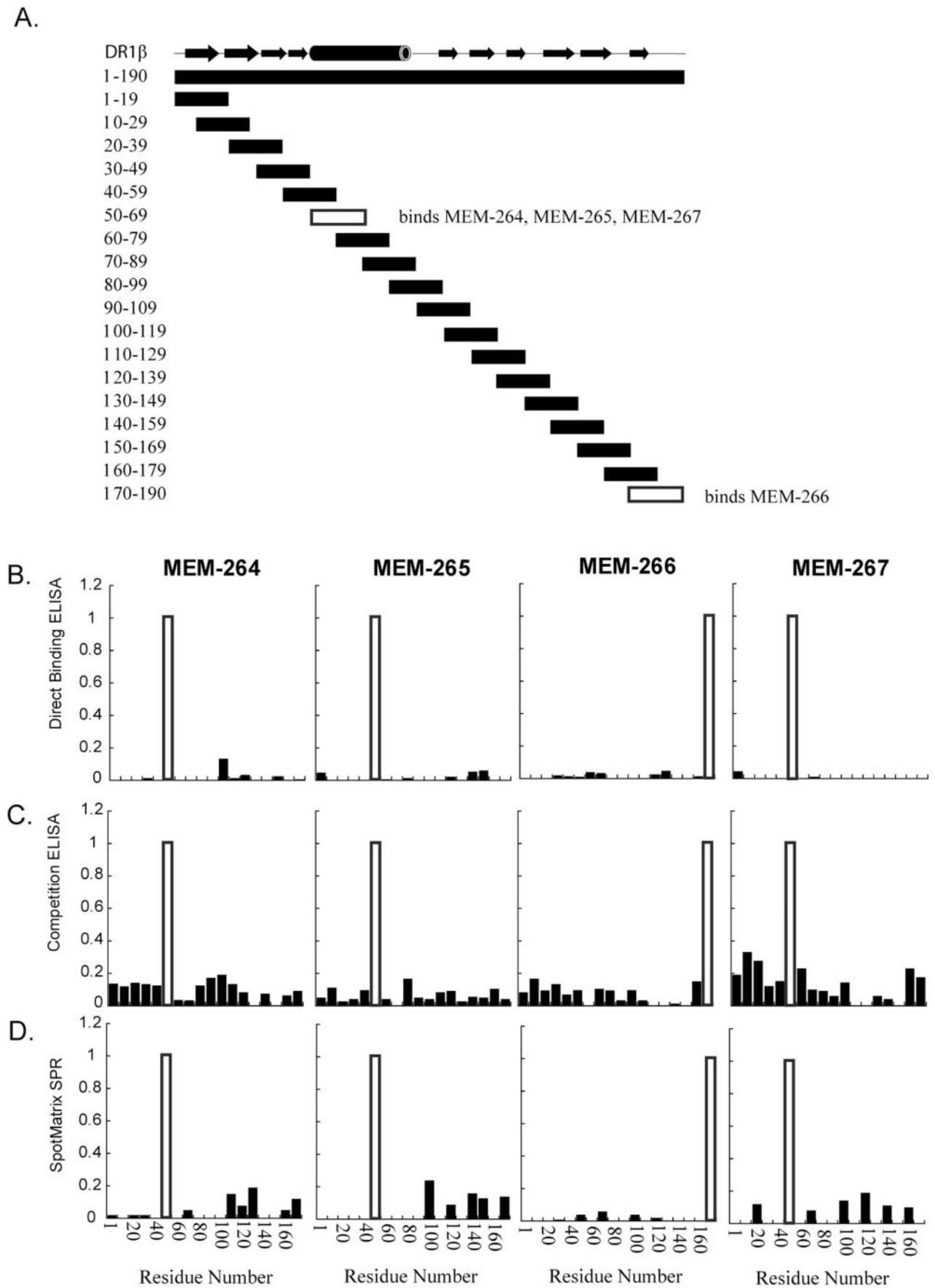

FIG. 5. **Epitope mapping of MEM antibodies.** *A*, mapping strategy using overlapping 20-mer peptides (overlap of 10 amino acids) spanning the entire DR1 sequence. Each monoclonal antibody was tested by direct binding ELISA (*B*), by competition ELISA (*C*), and by SpotMatrix SPR analysis (*D*). For each antibody, a single peptide was observed to bind: MEM-264, MEM-265, and MEM-267 bind DRβ-(50–69), and MEM-266 binds DRβ-(170–190).

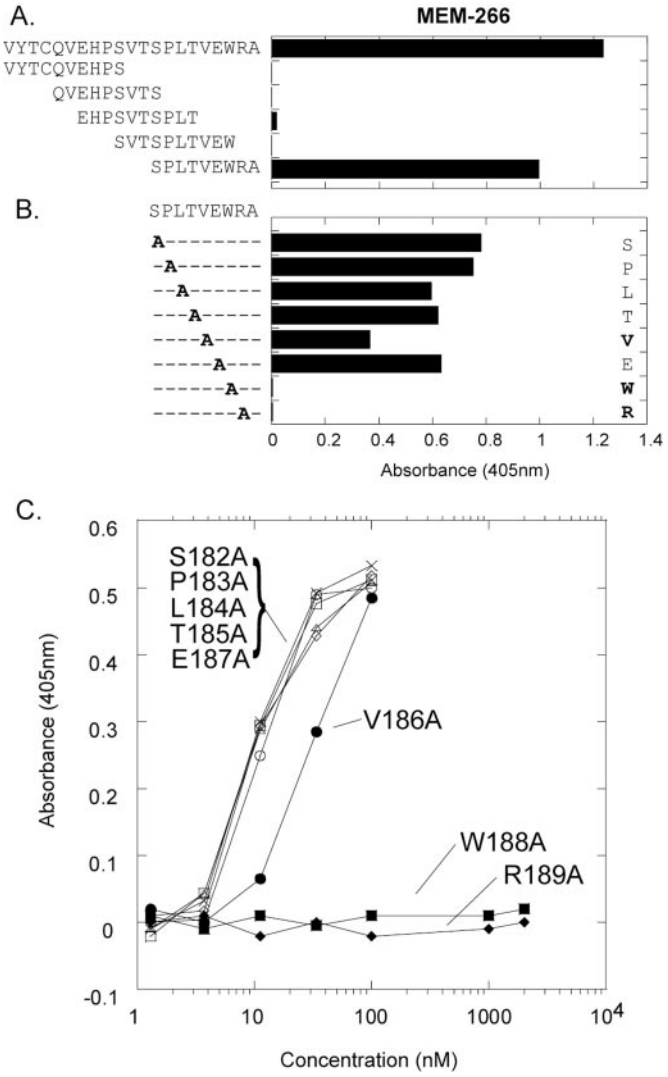

FIG. 6. **Fine mapping of the MEM-266 epitope within the peptide DRβ-(170–190).** *A*, overlapping 10-mer peptides used to further define the MEM-266 epitope within the peptide DRβ-(170–190). In a direct binding ELISA, the antibody bound only to the COOH-terminal peptide DRβ-(182–190). *B* and *C*, alanine scan of DRβ-(182–190) to find the amino acid residues important for binding to MEM-266. Antibody binding was blocked by substitution of Trp-188 and Arg-189 and reduced by substitution of Val-186.

265, and MEM-267 with DR1 and DR4, which share these residues, and their lack of binding to DR52a, which contains two substitutions in this region (D57V and Y60S).

DISCUSSION

The human class II MHC protein HLA-DR1 undergoes a distinct conformational change as it binds to its peptide ligand. Previous studies have shown that the empty protein has a larger, less compact structure than the peptide-bound complex (5, 8), and that peptide binding induces conformational changes in the empty protein detectable in the ultraviolet circular dichrosim (8) and NMR spectra.[3] In this study, regions of the protein that participate in the conformational change were identified. A panel of four conformation-sensitive monoclonal antibodies was generated that recognize the β subunit and specifically bind to empty but not peptide-loaded forms of the protein. The antibodies also differentiate the empty and peptide-loaded forms of both purified recombinant protein and native protein expressed on the surface of antigen-presenting dendritic cells. Two regions of the protein that change upon peptide binding were identified (Fig. 8). One site, DR1β-(53–67), shown in *red* in Fig. 8*A*, is on the β subunit helical region near the peptide binding site, and overlaps with a similar site previously identified by an antibody raised against I-A^s (8, 55). A second site, DR1β-(186–189), shown in *cyan* in Fig. 8*A*, is remote from the peptide binding site at the bottom of the immunoglobulin-like domain.

The DR1β-(53–67) epitope is recognized by three of the antibodies, MEM-264, MEM-265, and MEM-267. The key residues for all of the antibodies are the same: Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67. These residues are spaced three to four residues apart in a pattern characteristic of a helical repeat. In an α helical model for this region, these residues would all fall in a contiguous region on one face of the helix. However, in structures of DR-peptide complexes, this region includes two kinks, such that the key residues are not found in a contiguous region. The side chains of Leu-53 and Asp-57 are exposed on a different face of the protein than the other residues, and Ser-63 lies on the other side of the helix from Tyr-60, Trp-61, and Leu-67 (Fig. 8*B*). This distributed, non-contiguous location of the key epitope residues in the structure of the peptide complex indicates that conformational changes in this region would be required to bring the key residues together in the empty protein and allow engagement by MEM-264, MEM-265, or MEM-267. Thus, these antibodies appear to be sensitive to conformational rearrangement(s) in this region, rather than to a simple steric block of key residues by peptide binding. This idea is supported by the loss of MEM recognition upon YRAL peptide binding, this peptide is likely to bind only in the P1–P4 region of the peptide binding site (56), far away from the DR1β-(53–67) epitope, with a closest approach of ~9 Å (from the peptide COOH terminus to Trp-60). Some evidence for conformational lability in this region of HLA-DR1 can be seen in crystal structures of its peptide complexes, which exhibit significant peptide-to-peptide variation in this region, and relatively high thermal B-factors, particularly for residues 63–67. Based on the pattern of key residues within the epitope, the epitope region is probably recognized by the MEM antibodies in a simple helical conformation distinct from that observed in the crystal structures, as this would place the key residues in position to interact with the antibody combining site. Whether this new conformation is induced upon antibody binding or present in the empty MHC protein is not clear, although we note that all of the antibodies that recognize this epitope bind to the empty protein much more tightly than to the free epitope peptides, consistent with the structure of the epitope region in the empty protein being similar to that recognized by the antibody.

The fourth antibody, MEM-266, shows the same conformational sensitivity as the other antibodies, but its epitope region is located at the COOH-terminal end of the DR1 extracellular domain, and includes the last five residues in the β strand at the bottom of the immunoglobulin-like domain. The key epitope residues, Trp-188 and Arg-189 (and to a lesser extent Val-186) are located in a contiguous, linear epitope, and MEM-266 exhibits similar affinities for the empty protein and the corresponding epitope peptides. Thus, this epitope is most likely non-conformational, with differential antibody binding regulated by accessibility changes to this region of the protein, rather than by local rearrangements. These residues are solvent-accessible in the structures of HLA-DR1 peptide complexes, but located in a cleft between the domains, and antibody accessibility in the peptide complex might be limited by steric interactions with other overhanging regions. In this sce-

[3] Z. Zavala-Ruiz, H. Schwalbe, and L. J. Stern, unpublished observations.

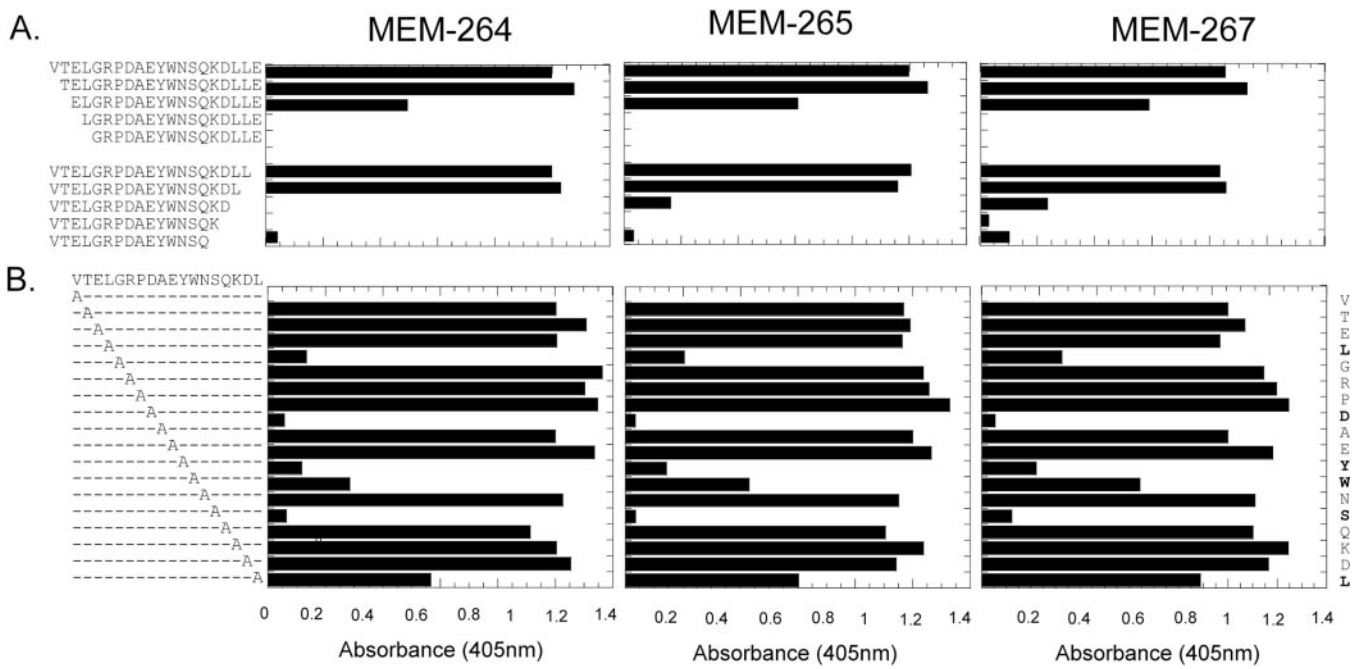

FIG. 7. **Fine mapping of MEM-264, MEM-265, and MEM-267 epitopes within the peptide DRβ-(50–69).** *A*, NH_2- and COOH-terminal deletions of the binding 20-mer peptide DRβ-(50–69) tested in an attempt to find a short minimal epitope for binding. Removal of more than two NH_2- or COOH-terminal residues abrogated binding activity, as observed using a direct-binding ELISA. *B*, alanine scan of the 18-mer peptide DRβ-(50–67) to find the amino acid residues important for binding to MEM-264. Antibody binding was blocked by substitution of residues Leu-53, Asp-57, Tyr-60, Trp-61, Ser-63, and Leu-67.

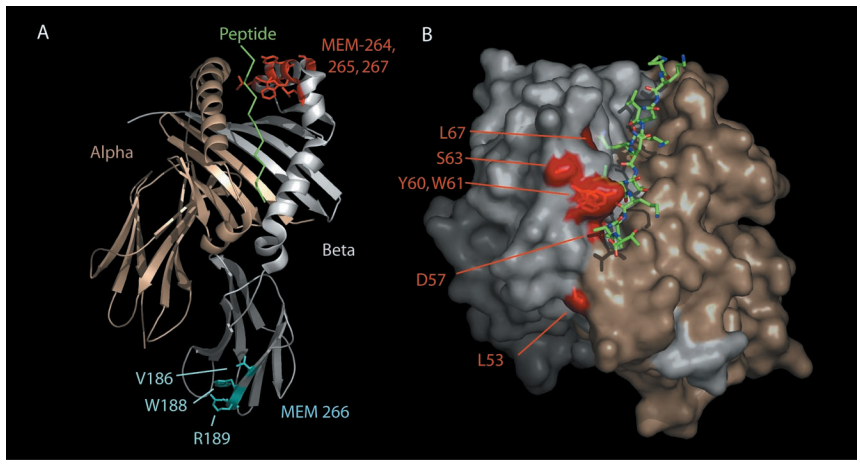

FIG. 8. **MEM epitope map onto the DR1-Ha structure.** *A*, ribbon diagram of HLA-DR1 bound to the HA-peptide. Important residues for binding of MEM-264, MEM-265, and MEM-267 are shown in *red*. Residues important for binding to MEM-266 are shown in *cyan*. *B*, close-up surface view of MEM-264, MEM-265, and MEM-267 epitopes viewed from the opposite face as *panel A*. Note that the side chains of important residues are not located on a contiguous face of the protein. Leucine 53 is located below the peptide binding groove and this region would appear to have to rearrange to bind to the MEM antibodies. Figures were generated using the program Pymol (The Pymol Molecular Graphics System, DeLano Scientific, San Carlos, CA; www.pymol.org).

nario, peptide binding would induce changes in the relative orientation of these domains, and expose Trp-188 and Arg-189 to MEM-266 binding. Rigid body shifts of the entire β2 domain, including rotations of up to 15 degrees, are routinely observed in comparisons of HLA-DR1 peptide complexes in different crystal forms (57). These realign the β2 domain relative to the peptide binding domain, and could potentially couple peptide binding to domain rotation via interactions between loop residues at the top end of the β2 domain and the underside of the P1 pocket (58).

Thus, the conformational changes associated with peptide binding are distributed throughout the protein. In fact, the MEM-266 epitope is the region of the extracellular domain furthest from the peptide binding site. What could be a physiological role for such a large conformational change? One possibility might be that the conformational changes regulate interactions with HLA-DM, the peptide-exchange catalyst required for efficient intracellular loading of peptides on class II MHC molecules. HLA-DM helps catalyze peptide binding and release through conformational effects on DR1 (35) and several models of interaction have been proposed (11, 13, 18, 35, 59, 60). Many of these models involve HLA-DM recognizing or promoting a structural change in HLA-DR that could facilitate peptide release. Recently, a screen for MHC mutants that disrupt DM activity has identified a number of putative DM-DR interaction sites, all on the same lateral face of the MHC proteins (61). Surprisingly, this set included V186K, which is located well away from the peptide binding site (61), and is part of the epitope region for MEM-266. Together, these results suggest that peptide-induced conformational changes propagate from the peptide binding site to the distal end of the β subunit, and conversely, that interprotein interactions in this region can be transmitted to the peptide binding site to regulate peptide release and binding kinetics.

In summary, a small panel of monoclonal antibodies able to detect peptide binding to HLA-DR1 and related human class II MHC alleles was produced. These antibodies can be used to investigate peptide loading processes *in vitro* or *in vivo*, and

have already provided important information on the location and extent of the peptide-induced conformational change in HLA-DR1.

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REFERENCES

1. Watts, C. (1997) *Annu. Rev. Immunol.* **15,** 821–850
2. McFarland, B. J., and Beeson, C. (2002) *Med. Res. Rev.* **22,** 168–203
3. Jardetzky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 734–738
4. Stern, L. J., and Wiley, D. C. (1994) *Structure* **2,** 245–251
5. Sato, A. K., Zarutskie, J. A., Rushe, M. M., Lomakin, A., Natarajan, S. K., Sadegh-Nasseri, S., Benedek, G. B., and Stern, L. J. (2000) *J. Biol. Chem.* **275,** 2165–2173
6. Natarajan, S. K., Assadi, M., and Sadegh-Nasseri, S. (1999) *J. Immunol.* **162,** 4030–4036
7. Natarajan, S. K., Stern, L. J., and Sadegh-Nasseri, S. (1999) *J. Immunol.* **162,** 3463–3470
8. Zarutskie, J. A., Sato, A. K., Rushe, M. M., Chan, I. C., Lomakin, A., Benedek, G. B., and Stern, L. J. (1999) *Biochemistry* **38,** 5878–5887
9. Mason, K., Denney, D. W., Jr., and McConnell, H. M. (1995) *J. Immunol.* **154,** 5216–5227
10. Mason, K., and McConnell, H. M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 12463–12466
11. Sadegh-Nasseri, S., and McConnell, H. M. (1989) *Nature* **337,** 274–276
12. Sadegh-Nasseri, S., Stern, L. J., Wiley, D. C., and Germain, R. N. (1994) *Nature* **370,** 647–650
13. Beeson, C., and McConnell, H. M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 8842–8845
14. Beeson, C., Rabinowitz, J., Tate, K., Gutgemann, I., Chien, Y. H., Jones, P. P., Davis, M. M., and McConnell, H. M. (1996) *J. Exp. Med.* **184,** 777–782
15. Schmitt, L., Boniface, J. J., Davis, M. M., and McConnell, H. M. (1999) *J. Mol. Biol.* **286,** 207–218
16. Sadegh-Nasseri, S., and Germain, R. N. (1991) *Nature* **353,** 167–170
17. Jensen, P. E. (1990) *J. Exp. Med.* **171,** 1779–1784
18. Dornmair, K., Rothenhausler, B., and McConnell, H. M. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54,** 409–416
19. Rabinowitz, J. D., Vrljic, M., Kasson, P. M., Liang, M. N., Busch, R., Boniface, J. J., Davis, M. M., and McConnell, H. M. (1998) *Immunity* **9,** 699–709
20. Denzin, L. K., and Cresswell, P. (1995) *Cell* **82,** 155–165
21. Kropshofer, H., Vogt, A. B., Moldenhauer, G., Hammer, J., Blum, J. S., and Hammerling, G. J. (1996) *EMBO J.* **15,** 6144–6154
22. Joshi, R. V., Zarutskie, J. A., and Stern, L. J. (2000) *Biochemistry* **39,** 3751–3762
23. Goldberg, M. E. (1991) *Trends Biochem. Sci.* **16,** 358–362
24. Fedorov, A. N., Friguet, B., Djavadi-Ohaniance, L., Alakhov, Y. B., and Goldberg, M. E. (1992) *J. Mol. Biol.* **228,** 351–358
25. Frayser, M., Sato, A. K., Xu, L., and Stern, L. J. (1999) *Protein Expression Purif.* **15,** 105–114
26. Li, Y., Li, H., Martin, R., and Mariuzza, R. A. (2000) *J. Mol. Biol.* **304,** 177–188
27. Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1994) *Nature* **368,** 215–221
28. Cameron, T. O., Norris, P. J., Patel, A., Moulon, C., Rosenberg, E. S., Mellins, E. D., Wedderburn, L. R., and Stern, L. J. (2002) *J. Immunol. Methods* **268,** 51–69
29. Cochran, J. R., and Stern, L. J. (2000) *Chem. Biol.* **7,** 683–696
30. Bouvier, M., and Wiley, D. C. (1994) *Science* **265,** 398–402
31. Bouvier, M., and Wiley, D. C. (1998) *Nat. Struct. Biol.* **5,** 377–384
32. Boniface, J. J., Lyons, D. S., Wettstein, D. A., Allbritton, N. L., and Davis, M. M. (1996) *J. Exp. Med.* **183,** 119–126
33. Fahnestock, M. L., Tamir, I., Narhi, L., and Bjorkman, P. J. (1992) *Science* **258,** 1658–1662
34. Runnels, H. A., Moore, J. C., and Jensen, P. E. (1996) *J. Exp. Med.* **183,** 127–136
35. Zarutskie, J. A., Busch, R., Zavala-Ruiz, Z., Rushe, M., Mellins, E. D., and Stern, L. J. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 12450–12455
36. Gorga, J. C., Knudsen, P. J., Foran, J. A., Strominger, J. L., and Burakoff, S. J. (1986) *Cell Immunol.* **103,** 160–173
37. Fu, X. T., and Karr, R. W. (1994) *Hum. Immunol.* **39,** 253–260
38. Stratikos, E., Wiley, D. C., and Stern, L. J. (2004) *J. Immunol.* **172,** 1109–1117
39. Robinson, J., Waller, M. J., Parham, P., de Groot, N., Bontrop, R., Kennedy, L. J., Stoehr, P., and Marsh, S. G. (2003) *Nucleic Acids Res.* **31,** 311–314
40. Hansen, B. E., Andersson, E. C., Madsen, L. S., Engberg, J., Sondergaard, L., Svejgaard, A., and Fugger, L. (1998) *Tissue Antigens* **51,** 119–128
41. Scheirle, A., Takacs, B., Kremer, L., Marin, F., and Sinigaglia, F. (1992) *J. Immunol.* **149,** 1994–1999
42. Stockel, J., Meinl, E., Hahnel, C., Malotka, J., Seitz, R., Drexler, K., Wekerle, H., and Dornmair, K. (1994) *J. Biol. Chem.* **269,** 29571–29578
43. Scott, C. A., Garcia, K. C., Carbone, F. R., Wilson, I. A., and Teyton, L. (1996) *J. Exp. Med.* **183,** 2087–2095
44. Scott, C. A., Garcia, K. C., Stura, E. A., Peterson, P. A., Wilson, I. A., and Teyton, L. (1998) *Protein Sci.* **7,** 413–418
45. Stratmann, T., Apostolopoulos, V., Mallet-Designe, V., Corper, A. L., Scott, C. A., Wilson, I. A., Kang, A. S., and Teyton, L. (2000) *J. Immunol.* **165,** 3214–3225
46. Wu, S., Maslanka, K., and Gorski, J. (1997) *J. Immunol.* **158,** 3221–3226
47. Santambrogio, L., Sato, A. K., Fischer, F. R., Dorf, M. E., and Stern, L. J. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 15050–15055
48. Romani, N., Reider, D., Heuer, M., Ebner, S., Kampgen, E., Eibl, B., Niederwieser, D., and Schuler, G. (1996) *J. Immunol. Methods* **196,** 137–151
49. Geysen, H. M., Rodda, S. J., Mason, T. J., Tribbick, G., and Schoofs, P. G. (1987) *J. Immunol. Methods* **102,** 259–274
50. Gampfer, J. M., Samstag, A., Waclavicek, M., Wolf, H. M., Eibl, M. M., and Gulle, H. (2002) *Vaccine* **20,** 3675–3684
51. Maeda, H., Miyamoto, M., Kokeguchi, S., Kono, T., Nishimura, F., Takashiba, S., and Murayama, Y. (2000) *FEMS Immunol. Med. Microbiol.* **28,** 219–224
52. Matsushita, K., Nisizawa, T., Nagaoka, S., Kawagoe, M., and Koga, T. (1994) *Infect. Immun.* **62,** 4034–4042
53. Kabat, E. A. (1970) *Ann. N. Y. Acad. Sci.* **169,** 43–54
54. MacCallum, R. M., Martin, A. C., and Thornton, J. M. (1996) *J. Mol. Biol.* **262,** 732–745
55. LaPan, K. E., Klapper, D. G., and Frelinger, J. A. (1992) *Hybridoma* **11,** 217–223
56. Bolin, D. R., Swain, A. L., Sarabu, R., Berthel, S. J., Gillespie, P., Huby, N. J., Makofske, R., Orzechowski, L., Perrotta, A., Toth, K., Cooper, J. P., Jiang, N., Falcioni, F., Campbell, R., Cox, D., Gaizband, D., Belunis, C. J., Vidovic, D., Ito, K., Crowther, R., Kammlott, U., Zhang, X., Palermo, R., Weber, D., Guenot, J., Nagy, Z., and Olson, G. L. (2000) *J. Med. Chem.* **43,** 2135–2148
57. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J., and Wiley, D. C. (1988) *Nature* **332,** 845–850
58. Murthy, V. L., and Stern, L. J. (1997) *Structure* **5,** 1385–1396
59. Schmitt, L., Kratz, J. R., Davis, M. M., and McConnell, H. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 6581–6586
60. Chou, C. L., and Sadegh-Nasseri, S. (2000) *J. Exp. Med.* **192,** 1697–1706
61. Doebele, R. C., Busch, R., Scott, H. M., Pashine, A., and Mellins, E. D. (2000) *Immunity* **13,** 517–527
62. Sadegh-Nasseri, S., and Germain, R. N. (1992) *Immunol. Today* **13,** 43–46