Peptide Ligands That Bind IgM Antibodies and Block Interaction with Antigen

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Peptide Ligands That Bind IgM Antibodies and Block Interaction with Antigen

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We have selected a peptide-display phage library on IgM Abs and identified a panel of phage-expressing peptides that bind to IgM Abs in general, but not to Abs of other classes. A synthetic peptide corresponding to one of the displayed peptide sequences also binds to IgM Abs. The peptides bind to both soluble pentameric Abs and to monomeric cell-surface IgM. The phage-displayed and synthetic peptides inhibit the binding of IgM Abs to Ag. These peptides may create confounding artifacts when IgM Abs are used for epitope mapping studies. Nonetheless, the peptides may have both experimental and therapeutic utility. The Journal of Immunology, 1999, 163: 826–833.

The use of bacteriophage display peptide libraries (PDPL), termed epitope or “mimotope” libraries, greatly facilitates identifying peptide ligands for proteins of interest (for reviews, see Refs. 1 and 2). Inspired by advances in peptide synthesis (3), Smith and colleagues pioneered PDPL construction in which vectors were designed for expression of random peptides fused to coat proteins, usually pII or pVIII, of filamentous phage (2). Thus, a multitude of different oligopeptides are surface displayed by the viral particles and available for interaction and affinity selection with a ligate of choice. DNA sequence information from the selected clones reveals the sequence of the displayed peptide. Most applications of PDPL technology have focused on protein-to-protein interactions. However, recent data from various labs have indicated the feasibility of finding peptide structures that mimic nonproteinaceous ligands such as biotin (4) or oligosaccharide epitopes (5–8).

We applied phage-display technology to search for structural equivalents of microbial polysaccharide epitopes. We had previously identified carbohydrate epitopes from Candida albicans and group B streptococci (GBS) that elicit protective Ab responses, as well as other carbohydrate epitopes that elicit nonprotective Abs. The Abs used to select the phage were of the IgM class, which are typical of T-independent, anti-carbohydrate responses. While using these IgM Abs to select for carbohydrate-mimetic phage (8), we identified a population of phage that bind to all IgM Abs regardless of antigenic specificity, presumably recognizing determinants in the constant regions. We characterized these phage and demonstrated, surprisingly, that the phage and peptides derived from them can inhibit the interaction between Ag and IgM Ab.

Materials and Methods

J404 nonapeptide PDPL

The J404 PDPL we used in our studies was constructed by one of the authors (J.B.B.) and is described elsewhere (1, 9, 10). The library displays random nonamer peptides from the N-terminal portion of the pIII capsid protein of kanamycin-resistant, filamentous bacteriophage M13K8Bt. The J404 library contains an estimated 5 × 10^10 different nonapeptides at high titer (1 × 10^13 pfu/ml).

Abs

Anti-Candida IgM mAbs B6.1 and B6 were isolated as previously described (11) and produced in serum-free medium (BG 101 Liquid Kit; Irvine Scientific, Santa Ana, CA). The Abs were concentrated by ammonium sulfate precipitation and exhaustively dialyzed against Dulbecco’s PBS (DPBS) (Sigma, St. Louis, MO). Anti-GBS IgM mAbs S7, S9, and S10 (12) were prepared as mouse ascites, and the IgM fraction was isolated by distilled water dialysis. S7 and S9 are directed against carbohydrate Ags, while S10 is specific for the GBS Ibc protein. Monoclonal anti-GBS mAbs mAbs B6.1 and 2A6 are specific for the GBS type III capsular polysaccharide (8), as is the IgM S9 (12). Additional mAbs and polyclonal Abs were used to assess peptide binding and Ig class specificity. Murine IgM mAbs used included: H9 and C6 (13); mt9A, mt5A, mt6F, F3 × 63 (14); 2B3.1, A4.1, C3.4, JD3 (M. Rieselman and J.E.C., unpublished observations), and T17 directed against the synthetic peptide (Y-E)-A-K- (15). Murine IgG mAbs included 6CS-H4 (a gift from Kevin C. Hazen, University of Virginia, Charlottesville, VA), 44.1 (9), and 924 (16). Polyclonal prepa-

Preparation of selection matrices

Ab affinity matrices for interaction with the J404 PDPL were mAb B6.1 adsorbed to polystyrene dishes, mAb B6.1 conjugated to Sepharose 4B (CL-4B-200; Sigma), or mAb S10 conjugated to Sepharose beads. Polystyrene dishes (Falcon 35 mm; Becton Dickinson, San Diego, CA) were coated for 2 h at room temperature with 1 mg mAb B6.1/ml DPBS and washed multiple times with cold DPBS. For mAb-conjugated Sepharose matrices, the Sepharose 4B was activated with cyanogen bromide as previously described (17) and coated with mAb B6.1 or S10 (3 mg per ml packed beads, 16 h at 4°C), washed, and blocked in 1% BSA (ICN Biomedicals, Aurora, OH) in DPBS before incubation with phage.

Interaction of the J404 library with IgM mAb matrices

All Ab-coated dishes and beads were preblocked for 1 h in DPBS plus 1% BSA before incubation with the library. Three independent selections (mAb B6.1-dish, MAb B6.1-Sepharose, and mAb S10-Sepharose) were performed with aliquots from the J404 PDPL. Manipulation of the nonapeptide PDPL and appropriate Escherichia coli K91 host cells were essentially as described (9). Briefly, aliquots of the nonapeptide PDPL...
Ags were conducted by coating microtiter wells with 0.5 mg/ml of the cell wall extract (19), blocking 1 h with 1% Ficoll 400 in TBS, and then blocking 2 h in BLOTTO. The inhibition assay was performed by either preincubating the Ab with phage or by adding the phage to microtiter wells after the Ab solution was present. For the preincubation inhibition assays, mAbs were diluted (0.25 μg/ml) in BLOTTO alone or mixed with 1.8 × 10^7 phage/ml and incubated for 1 h before placing 100 μl of the mixture into the cell wall extract-coated wells (2 h). For the later addition of phage, mAbs were incubated in wells for 1 h, and then phage was added to the microtiter wells and additionally incubated for 1 h. Wells were washed, and an HRP-conjugated goat anti-mouse polyvalent Ig was added and incubated 2–4 h. Substrate containing O-phenylenediamine, H_2O_2, in 0.1 M sodium citrate, pH 5.0, was added, and the color was developed for 10–30 min. Reactions were stopped by the addition of 10% H_2SO_4, and A_405 was measured.

GBS were coated onto microtiter wells using poly-l-lysine and glutaraldehyde as described elsewhere (8). Plates were blocked with 1% BSA. IgM anti-GBS Abs were mixed with phage or with KLH-conjugated peptides derived from the phage and incubated in microtiter wells at 4°C for 16 h. The plates were washed and incubated with AP-conjugated anti-Ig for 6 h, followed by washing and the addition of p-nitrophenyl phosphate. A_405 was then determined.

Latex agglutination for measurement of rheumatoid factor (RF)

Latex beads coated with aggregated IgG were obtained from REFSSCAN Kit (Becton Dickinson, Cockeyville, MD). Serum containing high titered RF activity were either contained within the kit or were obtained from the Division of Rheumatology, University of Utah School of Medicine (Salt Lake City, UT). Pretreated dilutions of serum were premixed with phage and then added to the latex beads. The mixture was stirred and allowed to incubate with slow shaking for 20 min before photography.

Flow cytometry for cell surface IgM

Murine B lymphoma cells expressing cell surface IgM (designated SFS) or differentiated to express IgA (4F10) (20, 21) were incubated with phage in PBS/BSA/0.1% sodium azide, washed, incubated with rabbit anti-M13 antisera, washed, and then stained with FITC-conjugated anti-rabbit Ig (Cappell Laboratories, Durham, NC). Alternatively, cells were directly stained with FITC-conjugated anti-mouse IgM (Cappell).

Preparation of synthetic peptide–carrier protein conjugate

The nonapeptide displayed by phage clone ed1 was chosen for conjugation to KLH with a heterobifunctional cross-linker, mmaleimidobenzyo-N-hydroxy-succinimide ester (MBS) (Pierce, Rockford, IL). A synthetic peptide containing a 4-aa extension including cysteine for cross-linking (YWVIPSSAWGPPC) was made (Bio-Synthesis, Lewisville, TX). Briefly, 10 mg KLH (Sigma KLH; Pierce) in degassed, nitrogen-sparged 0.05 M citrate-phosphate buffer, pH 5.0, was stirred gently with 2 mg cross-linker for 1 h at room temperature under a N_2 cap, then passed over a Sephadex G-25 column to separate the carrier-protein linker product from unreacted cross-linker. The MBS-KLH was placed into a fresh glass tube and stirred 6 h (N_2 cap, room temperature) with 5 mg of the synthetic ed1 peptide, which was dissolved in 50 μl dimethyl formamide. The sample was dialyzed against 3 L of the citrate-phosphate buffer, pH 5.0, to remove free peptide and then dialyzed against three changes of DPBS. Aliquots of the ed1-KLH-conjugate preparation were stored at −20°C before use.

Results

Independent IgM selections enrich for phage expressing similar peptide sequences

Phage were selected in four distinct selections on three different mAbs, immobilized either on Sepharose beads or on polystyrene dishes. In each case, the final selection pools contained phage clones that bound to multiple IgM Abs in ELISA or immunoblot analysis (see below). Nonapeptide sequences from individual IgM-binding phage clones are shown in Table I. Phage displaying some sequences, especially those designated ed1, ed4, and ps1, were isolated considerably more frequently than the others. All sequences contain a tryptophan residue in the C-terminal or penultimate position, and the sequences shown in Table I are aligned to that residue. While there is variability in the sequences capable of binding to IgM, certain motifs are strongly represented, such as aspartate on the amino-terminal side of the conserved tryptophan and W I S P / S Q X D W in many clones. The absolute or minimal sequence requirements necessary for IgM binding activity have not been established.

Binding of phage and peptide to IgM Abs

We have used immunoblotting and ELISA to demonstrate binding to IgM by phage and peptides derived from the phage. Phage clones were initially identified as binding to IgM by plaque lift immunoblots (data not shown).

Preparation and analysis of immunoblots

SDS-PAGE, transfer of proteins, and immunoblot preparations were essentially as described (18). Briefly, phage samples were electrophoresed in 12.5% polyacrylamide gels, and proteins were transferred to nitrocellulose membranes (BA83; Schleicher and Schuell, Keene, NH) with a semidry blotting system (MiliBlot-SD; MilliPore, Bedford, MA). NCM were briefly rinsed in transfer buffer and dried. For immunoblotting, membranes were rehydrated in water, washed for 10 min in DPBS, blocked (1–2°C) for 2 h in a fresh solution of DPBS containing 5% (w/v) nonfat milk and 0.1–0.2% Tween 20 (BLOTTO). BLOTTO was used for all Ab dilutions and wash steps. Blocked membranes were incubated overnight (4°C) with monoclonal (5 μg/ml or a 1:200 dilution of ascites preparations) or polyclonal Abs (1:200 dilution of serum samples). Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs. Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs. Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs. Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs. Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs. Blots were washed three times (10 min each) and incubated with alkaline phosphatase (AP) or HRP-conjugated secondary Abs.
Immunoblots of SDS-PAGE-separated phage proteins were used to demonstrate that IgM binding by phage involves the chimeric pIII-displayed peptide and also to show the specificity of binding. IgM-binding or nonbinding irrelevant phage were subjected to SDS-PAGE, blotted onto nitrocellulose, and were incubated with either one of two different IgM mAbs or a murine IgG mAb. The results of the immunoblot with the IgM mAb B6 is shown in Fig. 1. Signal occurred at the appropriate mobility for the pIII-nonapeptide chimeric protein in the lanes containing the IgM-binding phage (lane 4, es81; lane 6, ed9; lane 7, ed23; lane 8, ed36; lane 9, ed42; and lane 17, ed1), but not with any of the irrelevant phage clones (lanes 2, 3, 5, and 10–16) or with the parental phage M13KBst (lane 18). Duplicate blots were prepared and tested against a second IgM mAb, B6.1, or an IgG mAb, 6C5-H4. The identical pattern was seen with the IgM, and no reactivity was seen with the IgG (data not shown).

**FIGURE 1.** mAb B6 reactivity to pIII-displayed peptides from various phage clones. *Lane 1* was stained with amido black to show the proteins contained in the high molecular mass standards. Lanes 2–18 contain the following phage clones blotted onto NCM: lane 2, es109, displayed sequence RDVAKHKSMY; lane 3, es10 QQQKYGWTS; lane 4, es81 DWIPQASWE; lane 5, es40 NTRGMDWVE; lane 6, ed9 EABYSK-DWL; lane 7, ed23 AGIWQKDWL; lane 8, ed36 SWISSRDWT; lane 9, ed42 AGAIWQRDW; lane 10, nd1 GGIVARLTG; lane 11, ndb LHYVRSYN; lane 12, nd9 SAKDPLIGA; lane 13, nd2 AGQDWEWG; lane 14, ns4 KLRRAMHWD; lane 15, ns14 WVSKASTVW; lane 16, es29 YSSVPPGA; lane 17, ed1 YDWIPSSAW; lane 18, M13KBst library parent vector. The NCM was then incubated with IgM mAb B6. Binding of Ab B6 was detected with enzyme-conjugated anti-mouse IgM. Phage clones in lanes 2–17 were present in the final-round selection pool of selection on mAb B6.1. Only those in lanes 4, 6, 7, 8, 9, and 17 bound IgM.

**FIGURE 2.** Immunoblots of phage ed1 and irrelevant clone J508,22 probed with various Abs. Phage were run on SDS-PAGE gels and blotted onto nitrocellulose. Blot strips were incubated with various mAbs or polyclonal Abs and appropriate secondary Abs to determine Ig binding to the pIII-displayed peptides from clone ed1 and irrelevant clone J508,22. Clone ed1 displays peptide YDWIPSSAW, and clone J508,22 displays peptide AQPQVRPIG. Clone J508,22 was selected as an irrelevant control phage that represents the epitope of mAb 44.1, an IgG (9). In each panel, the migration point of pIII is labeled. A, Phage ed1 blot strips in lanes 1–13 were incubated with the following IgM mAbs: lane 1, B6.1; lane 2, 2B3.1; lane 3, H9; lane 4, A4.1; lane 5, C3.4; lane 6, JD3; lane 7, A6.1; lane 8, C6; lane 9, 10G; lane 10, m9A; lane 11, m5A; lane 12, m6F; and lane 13, P3 x 63. Lane 14 was incubated with a mouse anti-Candida polyclonal ascites, and lane 15 was incubated with a rabbit anti-Candida antiserum. Lanes 1–14 and control (no primary Ab) lane 16 were incubated with affinity-purified AP-conjugated goat anti-mouse-μ-chain-specific secondary Ab. Lane 15 and control (no primary Ab) lane 17 received affinity-purified HRP-conjugated goat anti-rabbit polyclonal Ig secondary Ab, B and C. Western blot strips of clone ed1 (B) and clone J508,22 (C) were probed with the following primary Abs: lane 1, IgM mAb S10; lane 2, IgM mAb T17; lane 3, IgG mAb 44.1; lane 4, IgG mAb 6C5-H4; and lanes 5 and 6, blocking buffer only. Secondary Abs were 1:1000 dilutions of affinity-purified AP conjugates: lanes 1, 2, and 5, goat anti-mouse-μ-chain-specific Ab; and lanes 3, 4, and 6, goat anti-mouse-γ-chain-specific Ab. D, Western blot strips of clone ed1. Lanes 1 and 3 were incubated with 1:500 human serum, while control lanes 2 and 4 were incubated in block alone. Secondary Abs were 1:1000 dilutions of affinity-purified AP conjugates: lanes 1 and 2, goat-human γ-chain-specific Ab; and lanes 3 and 4, goat anti-human-μ-chain-specific Ab.
Phage clone ed1 was chosen for further analysis. A preparative blot of the phage proteins was evaluated for reactivity with additional IgM Abs, including murine mAbs and polyclonal Abs from various species, as well as to IgG Abs (Fig. 2). All IgM mAbs bound the ed1 pIII-displayed nonapeptide (Fig. 2A, lanes 1–13). Because the mAbs in Fig. 2A were primarily directed against carbohydrate Ags, two mAbs against protein Ags, T17 and S10, were tested for binding to ed1. Fig. 2, B and C, lanes 1 and 2, demonstrate that these IgMs also bind to ed1, but not to an irrelevant phage. In lanes 3 and 4, we showed that two IgG mAbs are not bound by ed1 (Fig. 2B), but that one of the IgGs will bind to phage displaying the epitope recognized by that IgG (Fig. 2C). IgM Abs in a murine polyclonal ascites (Fig. 2A, lane 14) are bound by ed1. IgM Abs from human and rabbit serum samples also bound the pIII band (Fig. 2A, lane 15, and Fig. 2B, lane 3). The ed1 pIII did not bind IgG Abs, neither mAbs as described above, nor IgGs found in the affinity-purified secondary Abs used for the control strips (Fig. 2A, lanes 16 and 17, Fig. 2B, lanes 5 and 6, and Fig. 2D, lanes 1, 2, and 4).

**Table II. Binding of phage to IgM, but not to IgG**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Ab-Coating Wells*</th>
<th>None</th>
<th>924</th>
<th>S7</th>
<th>S9</th>
<th>B6.1</th>
</tr>
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<tbody>
<tr>
<td>ps55</td>
<td></td>
<td>0.24</td>
<td>0.39</td>
<td>0.31</td>
<td>0.21</td>
<td>1.83</td>
</tr>
<tr>
<td>S9-24</td>
<td></td>
<td>0.03</td>
<td>0.07</td>
<td>0.07</td>
<td>1.77</td>
<td>0.15</td>
</tr>
<tr>
<td>S7-B</td>
<td></td>
<td>0.05</td>
<td>0.1</td>
<td>1.74</td>
<td>0.16</td>
<td>0.2</td>
</tr>
<tr>
<td>S10-4</td>
<td></td>
<td>0.07</td>
<td>0.13</td>
<td>1.64</td>
<td>1.54</td>
<td>2.41</td>
</tr>
<tr>
<td>S10-8</td>
<td></td>
<td>0.03</td>
<td>0.08</td>
<td>1.61</td>
<td>1.86</td>
<td>2.31</td>
</tr>
</tbody>
</table>

* Wells were coated with IgM (S7, S9, B6.1), IgG (924) at 5 μg/ml, or no Ab.

ELISA was performed to demonstrate the specificity and avidity of phage binding. Specificity of binding of the phage to IgM Abs is shown in Table II. Two different IgM-binding phage (S10-4 and S10-8) were compared with three different Ab-specific phage (8) in an ELISA in which microtiter wells were coated with different Abs. S10-4 and S10-8 bound to wells coated with each of the IgM.

**FIGURE 3.** Binding of phage to IgM and IgG Abs. Microtiter wells were coated with the indicated mAbs. Phage (10⁹/well) were incubated with the mAbs. Unbound phage were washed out, and the remaining phage, bound to the Ab, were detected with anti-M13 Ab, enzyme-conjugated secondary Ab, and colorimetric substrate. The results are shown as A₄₀₅ (mean and SEM). S9 is an IgM Ab binding to a carbohydrate Ag, and S10 and T17 are IgM anti-protein Abs. Abs 924 and 41.1 are IgG.

**FIGURE 4.** Phage and peptide conjugates bind to anti-GBS mAb S9. A. Binding of phage to S9. ELISA plates were coated with mAb S9. Varying amounts of phage were added to the wells, incubated, and then washed. Phage binding was detected with rabbit anti-M13 antiseraum followed by AP-conjugated anti-rabbit Ig. The results are reported as A₄₀₅. B. Binding of peptide to S9. ELISA plates were coated with mAb S9. Varying concentrations of ed1-KLH conjugate, pep2 (WENWMMGNA)-KLH conjugate, or KLH were added to the wells, incubated, and then washed. Binding was detected with rabbit anti-KLH antiseraum followed by AP-conjugated anti-rabbit Ig. The results are reported as A₄₀₅.

**FIGURE 5.** Binding of phage to cell-surface IgM. Murine B cell lymphomas 5F5 (surface IgM⁺) and 4F10 (surface IgM⁻) were incubated with 3 x 10⁶ phage, washed, and incubated with rabbit anti-M13 antiseraum followed by FITC-conjugated anti-rabbit Ig (two left sets of panels) or were incubated only with an FITC-conjugated anti-mouse μ-chain (right). Labeled cells were analyzed by flow cytometry. Cell number is on the vertical axis, fluorescence intensity is on the horizontal.
Table III. Inhibition of Ab binding to GBS by phage

<table>
<thead>
<tr>
<th>Phage</th>
<th>S9</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>No phage</td>
<td>2.08</td>
<td>2.25</td>
</tr>
<tr>
<td>M13KBst</td>
<td>2.03</td>
<td>2.44</td>
</tr>
<tr>
<td>S10-4</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>S9-24</td>
<td>1.56</td>
<td>2.29</td>
</tr>
<tr>
<td>S7-B</td>
<td>2.10</td>
<td>0.48</td>
</tr>
</tbody>
</table>

a Binding of the indicated Ab (0.3 μg/ml) to ELISA wells coated with GBS was detected with AP-conjugated anti-mouse IgM.

b Abs were premixed with 10^10 of the indicated phage prior to addition to ELISA plate.

c A_105, mean of duplicate samples.

Inhibition of Ab binding by phage and phage-derived peptides

We next tested the ability of the IgM-binding phage to inhibit Ag binding by IgM Abs. We have performed these experiments in multiple different antigenic systems.

The ability of phage to block the binding to GBS of two anti-GBS mAbs directed against two different carbohydrate epitopes (12) is shown in Table III. For each mAb, the inhibition caused by the IgM-binding phage S10-4 was compared with the inhibition seen with mAb-specific phage described in a previous publication (8). Phage S10-4 inhibited both mAbs, the parental M13KBst inhibited neither, and each mAb-specific phage inhibited its respective mAb, but not the other. The inhibition obtained with S10-4 was equal or greater than that obtained with the mAb-specific phage, which presumably mimics the antigenic structure and binds within the Ab-combining site. Fig. 6 shows that both ed1 and S10-4 inhibit the binding of two IgM Abs, one specific for carbohydrate Ag (S9) and the other for a protein structure (S10), to GBS. However, they inhibit neither of two IgG Abs (2A6 and 1B1) with the same antigenic specificity as S9. The parental phage, M13KBst, does not inhibit Ag binding at all.

Because the phage inhibited the binding of IgM Ab to Ag, we sought to determine whether the phage bound within the combining site by performing a competitive inhibition, attempting to block the binding of phage to Ab S9 with purified GBS capsular polysaccharide. No inhibition was seen (data not shown).

The comparative ability of different IgM-binding phage to inhibit binding to GBS by mAb S9 is shown in Fig. 7A. The results

Indirect immunofluorescence and flow cytometry were used to demonstrate that IgM-specific phage bind to the monomeric cell-surface form of IgM (Fig. 5). Two different B cell lymphoma lines were used, one expressing cell-surface IgM (5F5), the other IgA (4F10). FITC-conjugated anti-mouse IgM Abs were used to demonstrate that 5F5 does express IgM and 4F10 does not (right panels). Binding of phage to the cells was detected by first incubating the cells with phage, washing, and then incubating with rabbit anti-M13 antiserum, followed by FITC-conjugated anti-rabbit Ig. As shown in the left panels of Fig. 5, S10-4 phage bind to the IgM-expressing cells, but not to those with cell-surface IgA. The parental phage, M13KBst, bind to neither (center panels).

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demonstrate that there are marked differences in the degree of inhibition by phage with different displayed sequences, and, not surprisingly, the relative inhibition correlates with the avidity of phage binding to the IgM Ab (Fig. 4A). We next tested whether Ab binding to Ag could be inhibited with a peptide corresponding to the sequence displayed by the ed1 phage (Fig. 7B). The results showed that the KLH-ed1 conjugate inhibited binding of Ab S9 to GBS, but neither KLH alone nor KLH conjugated to an irrelevant peptide had that effect.

The ability of IgM-binding phage to inhibit the interaction between IgM-anti-C. albicans Abs (19) to cell wall extract from C. albicans is shown in Fig. 8. As with the GBS, two different Abs with different epitope specificity were tested. Inhibition of both Abs, mAb B6.1 (Fig. 8A) and mAb B6 (Fig. 8B) was observed with phage ed1, but not with M13KBst. Much greater inhibition was seen when the phage and Ab were premixed and then added to Ag than when the phage were added after the initial Ag-Ab interaction had occurred.

Human RF is an anti-IgG autoantibody, primarily of the IgM class. Fig. 9 shows that ed1 phage, but not M13KBst, inhibited the RF-induced agglutination of IgG-coated latex beads.

IgM-binding phage do not inhibit complement activation

By use of ELISA, we tested the ability of phage to inhibit complement activation (22). IgM mAb and Ag were allowed to interact, then phage and human complement-containing serum was added. The binding of C1q and C3b to the immune complexes was assessed with specific antisera. There was no difference in complement binding in the presence of IgM-binding phage, control phage, or no phage (data not shown).

Discussion

In this report, we describe a set of peptides with IgM-binding activity. These peptides were inadvertently identified when we used a PDPL to select for peptides that mimic the Ags recognized by IgM Abs. These peptides bound to IgM Abs from mice, rabbits, and humans, but not to IgG Abs from those species. Binding activity was seen when the peptides were displayed as chimeras on the termini of the pIII protein on intact phage or when a synthetic peptide was conjugated to a carrier protein. IgM Abs specific for both carbohydrate and for protein Ags were bound. Unconjugated peptides were not tested because of insolubility in water at physiologic pH. Peptides bound to multimeric IgM and to the monomeric cell-surface form of IgM. Most surprisingly, the peptides inhibited the binding of Ag to IgM Abs. Inhibition of Ag binding...
was shown for five distinct Abs recognizing microbial carbohydrate and protein Ags and an aggregated protein autoantigen.

The site of peptide binding on the IgM Abs has not been defined. Because the peptides bind to all IgMs tested, but to no Abs of other classes, it is likely that the site is located within the H chain C regions. Because the peptides bind to IgM Abs of multiple species, presumably this site is well-conserved. Because the peptides bind to monomeric IgM, the J chain is an unlikely site of binding. Because of its effect on Ag binding, it is possible that the peptide binds the Fab. To determine whether these peptides represent a portion of a known IgM ligand, we performed a BLAST search using the National Center for Biotechnology Information website; no homologous protein sequences were identified. We have experimentally demonstrated that peptide binding does not affect complement activation, suggesting that the peptide binding site is not physically close to the C1q-binding site.

Although there is variability in the sequence of the different IgM-binding peptides, there are several well-conserved elements as well. A tryptophan is always found in the C-terminal or penultimate position, with an aspartate residue located on the N-terminal side of the tryptophan in the large majority of sequences. Interestingly, when there is not an aspartate adjacent to the C-terminal tryptophan, elsewhere in the sequence there is an aspartate-tryptophan set, suggesting that this may be a minimum requirement for IgM binding. Many of the residues are hydrophobic. Clearly the avidity of binding is influenced by the sequence of the displayed peptide (Fig. 4), with the sequence YDWIPSSAW (ed1) having the highest binding activity for murine IgM. Although we have shown that some of these peptides bind to IgM Abs of other species, we have not determined whether the sequence requirements for binding are the same for species other than mouse.

The mechanism whereby the peptides inhibit the binding of Ab to Ag is intriguing, because the peptides do not appear to bind within the Ab V region. Two potential mechanisms are steric hindrance or the induction of a conformational change in the IgM molecule that hinders the binding to Ag. Although steric hindrance has not been absolutely ruled out, for several reasons we do not believe it is the mechanism. The peptides bind to C-region determinants, and it is well established that anti-isotypic Abs do not block Ag binding. While the phage displaying the peptides are quite large and could conceivably cause steric inhibition over a large area, the peptides conjugated to KLH are smaller and less likely to sterically hinder. Finally, the stoichiometry of the interaction suggests that the mechanism is more than simple blocking of the Ag combining site on the IgM, because virtually complete inhibition of Ag binding was seen at ~1:1 molar ratio of displayed peptide to pentamer IgM (Fig. 7A). However, to definitively prove that the mechanism of inhibition is not steric, we will need to construct a small soluble peptide capable of binding to IgM and then test that molecule for inhibition of Ag binding. If steric hindrance does not account for the inhibition of Ag binding, then it is possible that the peptide either induces or prevents a conformational change in the IgM molecule. There is clear evidence that IgM molecules undergo a conformational shift upon Ag binding (23, 24), and it is presumed that this shift, a lifting of the Fab arms from the planar structure of the molecule, allows for greater accessibility of the Ag for the combining sites present on the large IgM molecule. Perhaps the IgM-binding peptides interfere with this process. Alternatively, the peptides might insert between unique CH1-CL1 interactions of the IgM and disrupt the Ag-binding site.

The IgM-binding peptides may represent potential artifacts when PDPL are used to epitope map IgM Abs, because such peptides appear to bind to an IgM Ab in a manner analogous to Ag. It is only when the specificity of binding and Ag inhibition are tested on irrelevant IgM Abs that the true nature of the peptide binding is revealed.

The peptides described in this publication may have both experimental and therapeutic utility. If the affinity of interaction between peptide and IgM molecule is sufficiently high, the peptides may be used to purify IgM Abs on affinity matrices. Because the peptides bind to cell-surface IgM, they may function as agents that activate B cells. The ability of the peptides to block the interaction between IgM Abs and Ag may be used to prevent deleterious Ag-Ab interactions or dissociate IgM-containing immune complexes. Such pathologic IgM Ab interactions can occur in hemolytic transfusion reactions, autoimmune diseases such as rheumatoid arthritis, and in paraneoplastic syndromes associated with Waldenstrom’s macroglobulinemia.

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References