

## Decorin-binding Sites in the Adhesin DbpA from *Borrelia burgdorferi*

A SYNTHETIC PEPTIDE APPROACH\*

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Lyme disease is caused by the spirochete *Borrelia burgdorferi* following transmission from infected *Ixodes* ticks to human hosts. Following colonization of the skin, spirochetes can disseminate throughout the body, resulting in complications that can include ocular, cardiac, neural, and skeletal disease. We have previously shown that *B. burgdorferi* expresses two closely related decorin-binding adhesins (DbpA and DbpB) of the MSCRAMM (microbial surface component recognizing adhesive matrix molecule) type that can mediate bacterial attachment to extracellular matrices in the host. Furthermore, three Lys residues in DbpA appear to be critical for the binding of DbpA to decorin. We have now characterized the interaction of DbpA and decorin further by using a synthetic peptide approach. We synthesized a panel of peptides that spanned the DbpA sequence and examined their ability to inhibit the binding of intact DbpA to decorin. From these studies, we identified a decorin-binding peptide that lost this activity if the sequence was either scrambled or if a critical Lys residue was chemically modified. A minimal decorin-binding peptide was identified by examining a set of truncated peptides. One peptide is proposed to contain the primary decorin-binding site in DbpA. By comparing the amino acid sequences of 29 different DbpA homologs from different *B. burgdorferi* sensu lato isolates, we discovered that the identified decorin-binding sequence was quite variable. Therefore, we synthesized a new panel of peptides containing the putative decorin-binding sequence of the different DbpA homologs. All of these peptides were active in our decorin-binding assay, and consensus decorin binding motifs are discussed.

Lyme disease is a chronic multisystemic disease caused by *Borrelia burgdorferi* sensu lato (1). *Borrelia*-infected ticks can transmit the bacteria to vertebrate hosts during blood meals

(1–3). After colonization of the dermis, spirochetes are able to disseminate to and affect a number of different organs. Initial symptoms often include headaches, malaise, and a rash at the infection site (erythema migrans). If antibiotic therapy is not administered at this time, dissemination of spirochetes may cause neurological, ocular, cardiac, and cutaneous disease in addition to arthritis (1–3). When correctly diagnosed and treated, disseminated Lyme disease generally can be prevented or resolved; however, up to 10% of patients are “treatment-resistant” (1, 4, 5). Treatment resistance in this patient population may be related to an autoimmune condition arising from cross-reactive epitopes between spirochete-expressed outer surface protein A and human lymphocyte function antigen 1 present on T cells (6, 7).

To further understand the disease process with the goal of developing a safe and effective Lyme disease vaccine, a characterization of *B. burgdorferi* surface proteins, in particular those required for adherence and the establishment of disease, is underway. Sequencing of the *B. burgdorferi* sensu stricto genome revealed ~130 putative surface lipoproteins, some of which are likely to be important in spirochete adhesion to host tissues (8, 9). To date, only a few mammalian ligands to specific *Borrelia* proteins have been identified including decorin, fibronectin, glycosaminoglycans,  $\beta_3$ -chain integrins, and complement regulator factor H (10–16).

Two decorin-binding proteins (Dbp),<sup>1</sup> DbpA and DbpB, were previously identified in our laboratory (14, 17). These surface-exposed ~20 kDa lipoproteins have amino acid sequences of ~40% identity and 51% similarity to each other in *B. burgdorferi* strain N40. Both Dbps bind decorin but with somewhat different affinity and/or specificity (14). A comparison of sequences from ~40 different *B. burgdorferi* sensu lato strains revealed that DbpB is a highly conserved protein (96–100% amino acid sequence identity) compared with the DbpA sequence that had between a 58 and 100% sequence identity among the strains analyzed (18).

Decorin, a molecule belonging to the small leucine-rich proteoglycan family, is found in the extracellular matrices of many tissues including the dermis and cartilage and is believed to play a role in regulating collagen fibril formation (19–21). It consists of a 38-kDa core protein substituted with an ~40-kDa glycosaminoglycan (GAG) chain as well as up to three *N*-linked oligosaccharides. The GAG chain, which is preferentially a

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<sup>1</sup> The abbreviations used are: Dbp, decorin-binding protein; MSCRAMM, microbial surface component recognizing adhesive matrix molecule; GAG, glycosaminoglycan; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; TBS, Tris-buffered saline; CD, circular dichroism; Dcn, decorin.



FIG. 1. **DbpA peptide sequences.** Peptide sequences spanning the length of DbpA from *B. burgdorferi* strain 297 (*underlined*). Lysine residues critical for decorin binding (Lys-82, Lys-163, and Lys-170) are shown in *boldface*.

dermatan sulfate chain in the skin, and a chondroitin sulfate chain in cartilage constitutes a highly negatively charged part of the molecule (22). Decorin together with biglycan, a structurally closely related proteoglycan substituted with two GAG chains and the recently discovered glycoprotein asporin, constitute the Class I subfamily of the small leucine-rich proteoglycan gene products (23–25). Although the DbpA- and DbpB-binding sites within decorin have not been well characterized, previous results indicated that both the decorin core protein and the GAG chain participate in this interaction (14).

We previously identified three conserved lysine residues (Lys-82, Lys-163, and Lys-170) in DbpA critical for the decorin binding activity of DbpA in a variety of assays (26). The current report describes a synthetic peptide approach to further characterize the binding site(s) in DbpA for decorin. We tentatively identify a short amino acid sequence spanning the Lys-82 region of DbpA as the decorin-binding site.

#### EXPERIMENTAL PROCEDURES

**Labeling of Decorin and Recombinant Proteins**—Decorin from bovine fetal skin provided by Dr. Lawrence Rosenberg (Montefiore Medical Center, New York, NY) was purified as described previously (27), stored in 4 M guanidine hydrochloride at  $-80^{\circ}\text{C}$ , and dialyzed extensively against phosphate-buffered saline (PBS) before use. Decorin, DbpA, or DbpB were labeled with either NHS-LS-biotin (sulfosuccinimidyl-6-(biotinamido) hexanoate) or with digoxigenin (digoxigenin-3-*O*-methyl-carbonyl- $\epsilon$ -aminocaproic acid-*N*-hydroxy-succinimide ester) as described previously (17) and stored at  $-20^{\circ}\text{C}$ .

**Synthesis of DbpA Peptides**—Peptides (Fig. 1) were synthesized by a solid phase method on a *p*-benzyloxybenzyl alcohol resin using *N*-(9-fluorenyl)methoxycarbonyl (Fmoc) chemistry and a model 396 Multiple Peptide Synthesizer from Advanced Chem Tech Inc. (Louisville, KY) as described previously (28). Peptides were purified using reverse-phase high pressure liquid chromatography on a Waters 625 liquid chromatography system using a  $\text{C}_{18}$  analytical column. The purity of the peptides was greater than 98%. Peptides were solubilized in PBS, pH 7.4, with up to 10%  $\text{Me}_2\text{SO}$  prior to use. Modification of lysine residues on DbpA-peptides was performed as described previously (26).

**Expression and Purification of Recombinant Proteins**—(His)<sub>6</sub>-tagged recombinant forms of DbpA (DbpA 549, amino acids 11–187) and DbpB

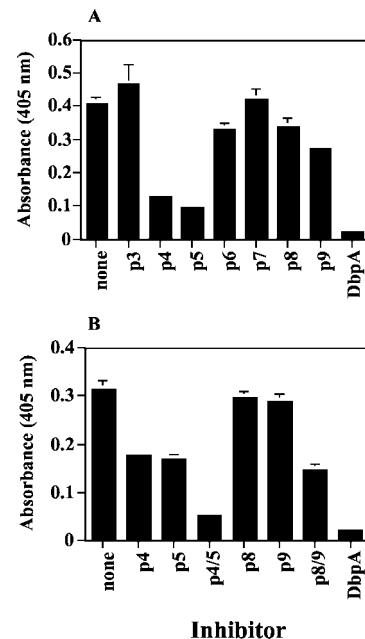
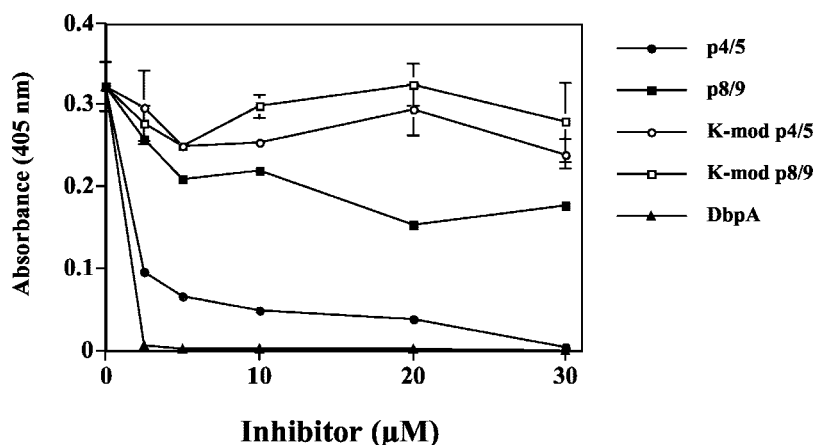


FIG. 2. **Inhibition of decorin binding by DbpA peptides.** Biotinylated decorin was preincubated with 20  $\mu\text{M}$  peptides 3–9 (A) or peptides 4, 5, 4/5, 8, 9, and 8/9 (B) for 1 h and then added to DbpA-coated microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples.

(DbpB 500, amino acids 21–187) from *B. burgdorferi* strain 297 were cloned into pQE-30 (Qiagen, Valencia, CA) and expressed in *Escherichia coli* JM101 (DbpA) or M15[pREP4] (DbpB) (17). Additional DbpA isoforms from *B. burgdorferi* strain HB19 (amino acids 10–189), *Borrelia garinii* strain Ip90 (amino acids 7–184), and *Borrelia afzelii* strain ACA-1 (amino acids 10–167) were cloned into pQE-30 and expressed in *E. coli* BL21 (HB19 and Ip90) or JM101 (ACA-1). Oligonucleotide primers used for PCR were as follows: 5'-CGCGGATCCATCTTTAATTTTAAAATTTACTT-3' (forward), 5'-GCGCTGCAGTTTTCATCAGTAAA-

**FIG. 3. Concentration-dependent peptide inhibition of decorin binding.** Biotinylated decorin was preincubated with DbpA, peptide 4/5, chemically modified peptide 4/5, peptide 8/9, or chemically modified peptide 8/9 at increasing concentrations for 1 h before being added to DbpA-coated microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples. *K-mod*, lysine-modified.

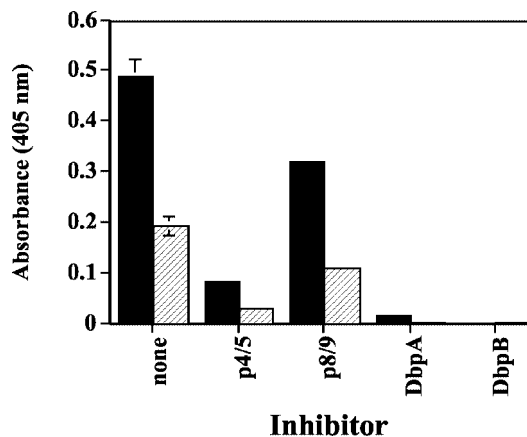


AGTAGAATTTTC-3' (reverse) for HB19; 5'-CGCGGATCCAATTTACT-TAACTAAGTTTAAATTG-3' (forward), 5'-AACTGCAGTTATTTAGGA-GTTTTGGCTTC-3' (reverse) for Ip90; and 5'-CGCGGATCCCTTACA-CTAAGTTTACTGCTAGC-3' (forward), 5'-GCGCTGCAGTTAGTTT-GTTTTCTTTTAAATGTTTC-3' (reverse) for ACA-1. *E. coli* was grown in Lennox Broth with the appropriate antibiotics, and recombinant protein expression was induced by the addition of isopropyl- $\beta$ -D-thiogalactopyranoside (0.2 mM, final concentration) (Invitrogen). Recombinant proteins were purified as described previously (26). The (His)<sub>6</sub>-tagged proteins were applied to a Hi-trap Ni<sup>2+</sup>-chelating column (Amersham Biosciences) and eluted with an imidazole gradient, dialyzed against PBS containing 10 mM EDTA, and then dialyzed against PBS and stored at -20 °C. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce).

**Inhibition Assay**—Immulon-1 microtiter plate wells (Dyner Technologies, Inc., Chantilly, VA) were coated with either 0.4  $\mu$ g of DbpA or DbpB or 1.0  $\mu$ g of decorin in 50  $\mu$ l of PBS overnight at 4 °C. The wells were washed and then blocked for 1 h with 200  $\mu$ l of Super Block (Pierce). During the blocking step, 0.06  $\mu$ g/ml biotin-conjugated decorin was pre-incubated at 37 °C with each DbpA-derived peptide, DbpA or DbpB, for the decorin inhibition assays. For the DbpA inhibition assay, 50  $\mu$ l of antiserum raised against DbpA, p4/5, or p8/9 was incubated with 50  $\mu$ l of digoxigenin-labeled DbpA (0.5  $\mu$ g) for 1 h. After washing, 100  $\mu$ l of the biotin-conjugated decorin/inhibitor mixture or 100  $\mu$ l of the conjugated DbpA-antiserum mixture was added to the wells and incubated for 1 h. The wells were washed and incubated with 100  $\mu$ l of alkaline phosphatase-conjugated streptavidin (1:3000 dilution) (Cooper Biomedical, Inc., Malvern, PA) for 1 h. After washing, 100  $\mu$ l of a 1 mg/ml Sigma 104 phosphatase substrate (Sigma) dissolved in 1 M diethanolamine, 0.5 mM MgCl<sub>2</sub>, pH 9.8, was added. Plates were washed four times with PBS, 0.05% Tween 20 in between all of the steps. All of the incubations were done at 37 °C, and all of the dilutions were made using Super Block. The absorbance at 405 nm was determined using a Thermomax microplate reader (Molecular Devices, Menlo Park, CA). The results are expressed as the mean  $\pm$  S.E. of triplicate samples minus the substrate control.

**Generation of Antiserum**—BALB/c mice (Harlan Sprague-Dawley, Indianapolis, IN) were immunized with 20  $\mu$ g of p4/5, p8/9, or recombinant DbpA emulsified in complete Freund's adjuvant as described previously (29). 4 weeks later, mice were boosted with the corresponding peptide/protein in Freund's incomplete adjuvant and serum was collected 1 week later (29). All of the animal procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University Health Science Center Institute of Biosciences and Technology.

**SDS-PAGE and Western Ligand Blot**—DbpA proteins were subjected to 12% SDS-PAGE under reducing conditions and either stained with Coomassie Brilliant Blue R250 (Sigma) or transferred to a nitrocellulose membrane. Additional protein-binding sites in the membrane were blocked with 10% nonfat dry milk in TBS (0.15 M NaCl, 20 mM Tris-HCl, pH 8.0). The membrane was washed in TBST (0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.05% Tween 20), incubated with digoxigenin-labeled decorin and alkaline phosphatase-conjugated anti-digoxigenin F(ab')<sub>2</sub> fragments (Roche Applied Science), and developed as described previously (17).



**FIG. 4. DbpA peptides 4/5 and 8/9 inhibit decorin binding to both DbpA and DbpB.** DbpA, DbpB, peptide 4/5, or peptide 8/9 at a concentration of 20  $\mu$ M were preincubated with biotinylated decorin and then added to DbpA-coated (black bars) or DbpB-coated (striped bars) microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples.

## RESULTS

**Peptide Inhibition of Decorin Binding to DbpA**—We previously reported that three lysine residues within the DbpA sequence (Lys-82, Lys-163, and Lys-170) were critical for the ability of DbpA to bind decorin (26). However, we do not know whether these Lys residues can potentially make contact with decorin and form part of the decorin-binding site in DbpA or whether they are required in DbpA for the MSCRAMM to adopt a decorin-binding conformation but are not contacting the ligand. To further define the decorin-binding site in DbpA, we now have used a synthetic peptide approach. Initially, nine (p1-p9) overlapping 25 amino acid-long peptides spanning the entire sequence of DbpA (Fig. 1) from *B. burgdorferi* strain 297 were synthesized and tested for their ability to inhibit the binding of biotin-labeled decorin to immobilized DbpA (Fig. 2A). In this assay, both peptides 4 and 5 (both containing Lys-82) had reproducible inhibitory effects on decorin binding to DbpA, although unlabeled DbpA was more potent and almost completely inhibited decorin binding (Fig. 2, A and B). Interestingly, peptide 8 (containing Lys-163) and peptide 9 (containing both Lys-163 and Lys-170) had only moderate inhibitory effects (Fig. 2, A and B). Peptide 1 was not included in this analysis because this region of DbpA contains a signal peptidase II recognition site and is therefore cleaved prior to lipid substitution (17). Because the cysteine residue in peptide 2 is used during lipid attachment and not normally exposed to the *in vivo* milieu during bacterial infection, this peptide also

FIG. 5. **The effect of peptide sequence scrambling on decorin inhibition.** Biotinylated decorin was preincubated with peptide 4/5A, scrambled (*scr*) peptide 4/5A, peptide 8/9, or scrambled peptide 8/9 at different concentrations for 1 h and then added to DbpA-coated microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples.

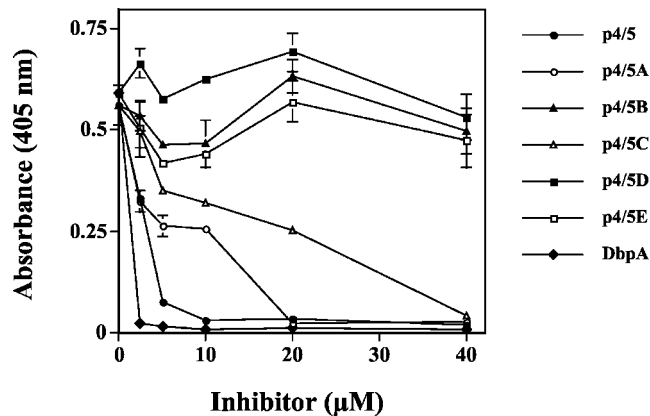
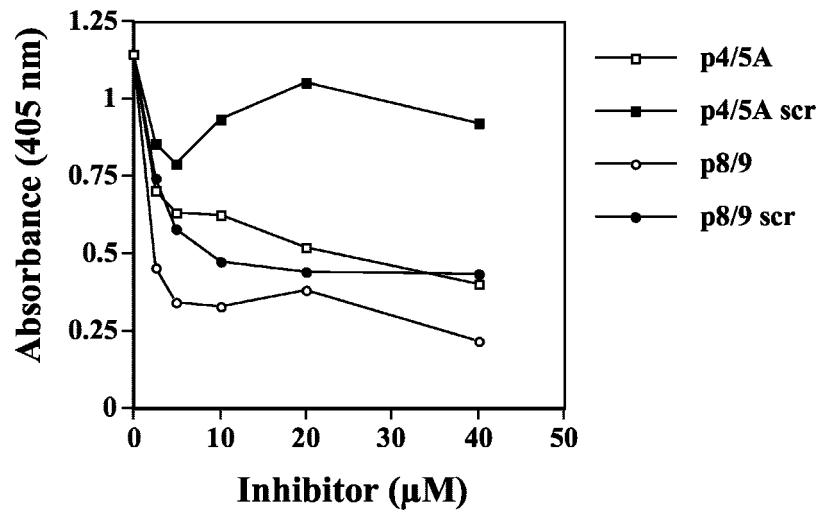


FIG. 6. **Truncated forms of peptide 4/5 retain inhibitory properties.** Biotinylated decorin was preincubated with DbpA, peptide 4/5, peptide 4/5A, peptide 4/5B, peptide 4/5C, peptide 4/5D, or peptide 4/5E at different concentrations for 1 h and then added to DbpA-coated microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples.

was not tested in depth, although preliminary data demonstrated that peptide 2 had no inhibitory activity (data not shown).

Two additional peptides, p4/5 and p8/9, containing Lys-82 and Lys-163/Lys-170 in central positions, respectively, (Fig. 1) were also synthesized and tested in the inhibition assay (Fig. 2B). Both p4/5 and p8/9 inhibited decorin binding more efficiently than peptides containing the critical lysine residues at either the C or N terminus as in peptides 4, 5, 8, and 9, respectively (Fig. 2B).

The importance of the lysine residues in peptides 4/5 and 8/9 for decorin binding was verified by chemical modification of the side chain amino groups in these residues (26). Native and chemically modified peptides 4/5 and 8/9 were examined for their ability to inhibit the binding of biotin-labeled DbpA to decorin in a concentration-dependent manner (Fig. 3). These results demonstrated that peptides subjected to chemical modification of lysine residues lost inhibitory activity. Furthermore, the concentration needed for 50% inhibition was less for p4/5 ( $ID_{50} < 2.5 \mu M$ ) than for p8/9 ( $ID_{50} \sim 20 \mu M$ ), and p4/5 but not p8/9 caused complete inhibition of DbpA binding at the highest concentration of peptide used. These results demonstrated that p4/5 is a more potent inhibitor of DbpA-decorin binding than p8/9 (Fig. 3).

**DbpA and DbpB Share Binding Site(s) in Decorin**—Since DbpA and DbpB bind decorin with a different affinity or spec-

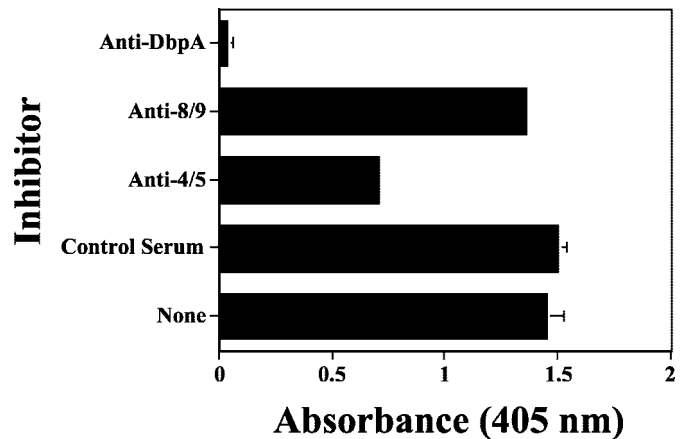


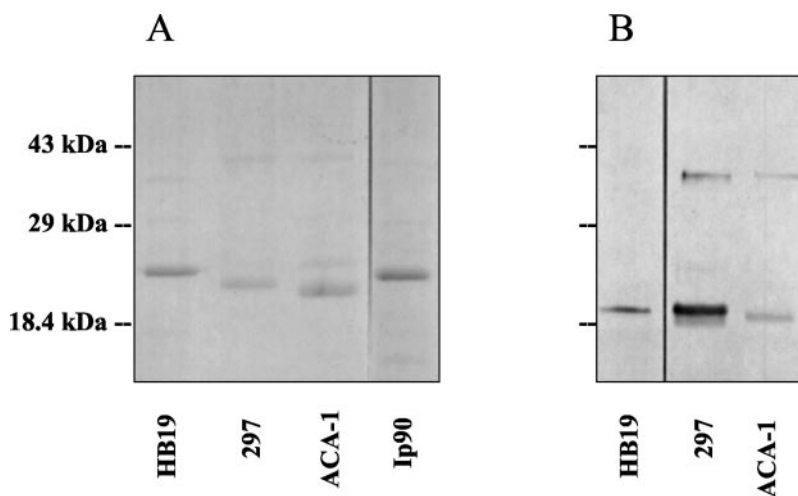
FIG. 7. **Reactivity of peptide antiserum against recombinant DbpA.** Digoxigenin-labeled DbpA was preincubated with antiserum raised against recombinant DbpA, p4/5, or p8/9 for 1 h before adding to decorin-coated microtiter wells. DbpA binding was quantified by ELISA, and the data are expressed as the mean  $\pm$  S.E. of the mean of triplicate samples.

ificity, we examined whether peptides 4/5 and 8/9 were equally efficient in inhibiting the binding of decorin to microtiter wells coated with either DbpA or DbpB (Fig. 4). DbpA peptides, DbpA or DbpB, at concentrations of  $20 \mu M$  were preincubated with biotinylated decorin prior to addition to either DbpA- or DbpB-coated wells. In the absence of inhibitor, decorin bound significantly better to DbpA than to DbpB, supporting the observation that decorin has a higher affinity for the former adhesin (Fig. 4). However, both p4/5 and p8/9 reduced decorin binding to either of the Dbps to a similar degree. Peptide 4/5 at  $20 \mu M$  reduced decorin binding by 83 and 84% to DbpA and DbpB, respectively. In the presence of p8/9 at the same concentration, decorin binding was reduced by 35 and 42% to DbpA and DbpB, respectively. Thus, peptides 4/5 and 8/9 are potent inhibitors of decorin binding to both DbpA and DbpB.

**The 4/5 Sequence Contains a Decorin-binding Site**—We scrambled the sequence of the p8/9 and a version of the 4/5 peptide (p4/5A, see below). The resulting peptides (Fig. 1, p4/5A *scr* and p8/9 *scr*) were tested in inhibition assays to determine whether the activity of the parent peptides were sequence-dependent (Fig. 5). Peptide 8/9 scrambled had a similar inhibitory activity compared with native p8/9, suggesting that interactions between p8/9 and decorin may be charge-dependent and a function of the relatively high number (six) of positively charged lysine residues in this peptide rather than the



**FIG. 8. SDS-PAGE and Western ligand blot analysis of recombinant DbpA isoforms.** Purified DbpA proteins were subjected to SDS-PAGE under reducing conditions and stained with Coomassie Brilliant Blue (10% gel) (A) or transferred onto a nitrocellulose membrane from a 12% gel (B). After blocking additional protein-binding sites, proteins on the membrane were probed with digoxigenin-labeled decorin and visualized by alkaline phosphatase reactivity.



result of the specific amino acid sequence of p8/9. Peptide 4/5 scrambled, in contrast, had essentially no inhibitory activity compared with native p4/5A. Since this peptide contains only a single lysine residue, we propose that the p4/5 inhibitory activity was dependent on a specific peptide sequence and that a decorin-binding site in DbpA is contained within the 4/5 sequence. If p4/5 contained a decorin-binding site, an antibody raised against this peptide should inhibit decorin binding to DbpA. Therefore, we generated and tested anti-p4/5 and anti-p8/9 sera for the ability to interfere with the binding of biotin-labeled DbpA to decorin. The anti-p4/5 serum reduced the binding of DbpA to decorin by 50% compared with an anti-serum raised against recombinant intact DbpA completely inhibited DbpA binding to decorin at the concentrations tested. Anti-p8/9 serum showed no significant inhibition at the concentrations tested (Fig. 7). These results are consistent with the hypothesis that the decorin-binding site is contained within the p4/5 sequence.

**A Minimal Binding Peptide Contains Lys-82**—We further examined the segment covered by the 4/5 peptide to define the minimal decorin-binding domain. This was done by synthesizing and testing five additional peptides (p4/5A-p4/5E) in inhibition assays (Fig. 1). Inhibition experiments revealed that p4/5A and p4/5C but not p4/5B, p4/5D, nor p4/5E inhibited decorin binding to immobilized DbpA (Fig. 6). The difference between the inhibition of decorin to DbpA by p4/5A (19 amino acids) and p4/5C (15 amino acids) ( $ID_{50} \sim 2.5 \mu M$  and  $ID_{50} \sim 10 \mu M$ , respectively) was 4-fold, but p4/5C had appreciable inhibitory activity, suggesting that the peptide 4/5C sequence (PFILEAKVRATTVAE) contains a full decorin-binding site. Peptides 4/5D and 4/5E, which were truncated by five or four residues at the C and N termini compared with p4/5C, respectively, were without activity, suggesting that the minimal decorin-binding sequence in the 4/5 region is represented by a central EAKVRA core sequence plus 1–5 flanking residues at both the N and C termini.

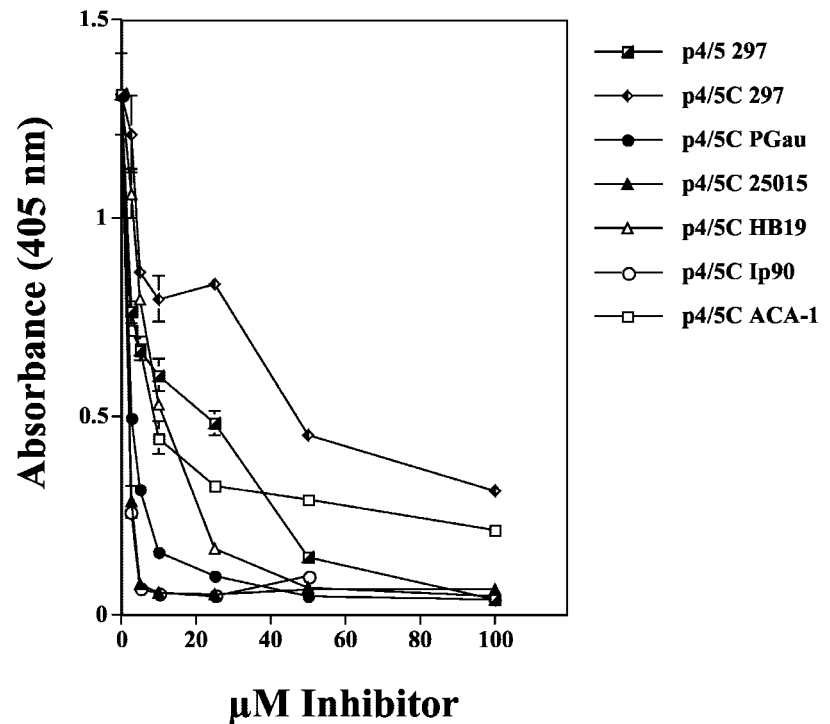
**DbpA Isoforms**—DbpA sequences from 29 *B. burgdorferi* sensu lato strains are between 58.3 and 100% identical to the DbpA amino acid sequence from strain 297 (18). Because of these sequence variations, we decided to examine the decorin binding activity of a selection of homologous DbpA proteins from different *Borrelia* strains. We expressed recombinant DbpA from one additional *B. burgdorferi* sensu stricto strain (HB19) (with an amino acid sequence that is 76.3% identical to DbpA from strain 297), one *B. garinii* strain (Ip90; 69.9% identity) and one *B. afzelii* strain (ACA-1; 62.7% identity). The generated recombinant DbpA proteins contained an N-terminal His tag and were purified by  $Ni^{2+}$ -chelating chromatogra-

<i>B. b.</i> 297	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> B31	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> 3028	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> HBNC	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> JD1	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> Lp4	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> Lp7	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> MC1	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> NCH-1	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> Sh-2-82	P-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> N40	S-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> HB19	S-F-I-L-E-A-K-V-R-A-T-T-V-A-E
<i>B. b.</i> ZS7	S-F-I-L-E-A-K-M-R-G-T-T-V-A-E
25015	P-F-I-L-K-A-K-M-R-A-I-E-V-A-E
<i>B. a.</i> PGau	P-F-I-I-K-A-K-I-R-T-T-S-V-A-L
<i>B. b.</i> G39/40	D-F-I-KQ-A-K-V-R-A-I-K-V-A-E
<i>B. b.</i> CA-3-87	D-F-I-KQ-A-K-V-R-A-I-K-V-A-N
<i>B. g.</i> VSBP	E-F-I-L-K-A-K-I-K-A-I-Q-V-A-E
<i>B. g.</i> G25	E-F-I-L-K-A-K-I-K-A-I-Q-V-A-E
<i>B. g.</i> PBr	E-F-I-L-K-A-K-I-K-A-I-Q-V-A-E
<i>B. g.</i> 20047	E-F-I-L-K-A-K-I-Q-A-I-Q-V-A-E
<i>B. a.</i> U01	E-F-I-L-K-A-K-I-Q-A-I-Q-V-A-G
<i>B. g.</i> Ip90	E-F-I-L-K-A-K-I-Q-A-I-Q-V-A-G
<i>B. g.</i> JEM4	E-F-I-L-K-A-K-I-Q-A-I-Q-V-A-K
<i>B. a.</i> VS461	S-Q-I-R-D-A-K-K-L-V-A-D-L-T-I
<i>B. a.</i> BO23	S-Q-I-R-D-A-K-K-L-V-A-D-L-T-I
<i>B. a.</i> IPF	S-Q-I-R-D-A-K-K-L-V-A-D-L-T-I
<i>B. a.</i> ACA-1	S-Q-I-R-A-A-K-I-R-V-A-D-L-T-K
<i>B. a.</i> M7	P-K-T-R-N-A-K-I-R-I-A-D-L-T-I
DbpB <i>B. b.</i>	L-A-L-R-E-A-K-V-Q-A-I-V-E-T-G
DbpB <i>B. g.</i>	S-A-L-R-E-A-K-V-Q-A-I-N-E-V-E
DbpB <i>B. a.</i>	L-A-L-R-E-A-K-I-Q-A-I-N-E-V-E

**FIG. 9. Alignment of DbpA and DbpB p4/5C amino acid sequences.** DbpA and DbpB amino acid sequences from different *Borrelia* strains (GenBank<sup>TM</sup>) were aligned along the 4/5C region. DbpB sequences are shown as consensus sequences for each *Borrelia* subspecies (based on alignment of 16 *B. burgdorferi* sensu stricto, four *B. garinii*, and three *B. afzelii* sequences). Amino acids identical to the 297 sequence are indicated in red. Peptides synthesized and used in inhibition experiments are denoted in a gray box. *B. b.*, *B. burgdorferi* sensu stricto; *B. a.*, *B. afzelii*; *B. g.*, *B. garinii*.

phy and analyzed by SDS-PAGE and Western ligand blot (Fig. 8). Monomers ( $\sim 20$  kDa) of all of the isoforms were visible on the Coomassie Blue-stained gel (Fig. 8A). In the Western ligand blot, decorin was able to bind to both monomers and dimers

FIG. 10. **DbpA 4/5C peptide isoforms inhibit decorin binding.** Biotinylated decorin was preincubated with peptide 4/5C isoforms at increasing concentrations for 1 h and then added to DbpA-coated microtiter wells, and decorin binding was quantified by ELISA. Data are expressed as the mean  $\pm$  S.E. of mean of triplicate samples.



(~40 kDa) of the 297, HB19, and ACA-1 isoforms (Fig. 8B). No decorin binding to the recombinant Ip90 isoform could be detected (data not shown).

Alignment of peptide 4/5C amino acid sequences from 29 different *B. burgdorferi* sensu lato strains showed most *B. burgdorferi* sensu stricto strains to have identical or very similar amino acid sequences in this area, whereas *B. garinii* and *B. afzelii* strains were more variable (Fig. 9). Also, DbpB consensus sequences showed some similarity to the 297 DbpA sequence in this region (Fig. 9). In addition to the lysine residue corresponding to Lys-82 in DbpA from *B. burgdorferi* strain 297, the adjacent alanine residue (Ala-81) was conserved throughout all of the DbpA and DbpB sequences examined (Fig. 9).

To further examine the role of the 4/5C region in decorin binding, we synthesized a panel of additional DbpA 4/5C homologous peptides corresponding to the 4/5C sequences of DbpA from *B. burgdorferi* HB19, group 25015, *B. garinii* Ip90, *B. afzelii* Pgau, and *B. afzelii* ACA-1. 4/5C peptides from these *Borrelia* strains are representative of the variability we observe among different p4/5C isoforms (Fig. 9). All of the p4/5C isoforms examined inhibited decorin binding to DbpA more effectively than either p4/5 or p4/5C from *B. burgdorferi* 297 (Fig. 10). One of the most potent inhibitory peptides was derived from strain Ip90 whose corresponding recombinant DbpA did not bind decorin in the Western ligand blot assay described above. Taken together, we concluded that all of the 4/5 regions in the DbpA proteins from different strains of *B. burgdorferi* sensu lato have the capacity to bind decorin and that this region forms a decorin-binding site in these proteins.

#### DISCUSSION

As *B. burgdorferi* is transmitted from an infected tick to a mammalian host, the spirochete must adapt to the new environment. This process cannot take place without a significant change in the protein-expression profile of the bacteria that will enable invading bacteria to colonize and disseminate in the mammalian host. *Borrelia* can turn on a group of genes encoding putative lipoproteins during the early stages of infection. This group includes the genes encoding the surface-exposed decorin-binding proteins, DbpA and DbpB (30–33). The ob-

served transcriptional regulation of the *dpb*-genes suggested that these MSCRAMMS play a role in the infection process. The importance of the DbpA-decorin interaction *in vivo* has been tested in mice deficient in decorin (*Dcn*<sup>-/-</sup>) (29). Compared with wild-type controls, *Dcn*<sup>-/-</sup> mice had fewer *Borrelia* positive tissues. Furthermore, arthritis incidence and severity were also reduced in *Dcn*<sup>-/-</sup> mice compared with *Dcn*<sup>+/+</sup> controls (29).

This work describes a detailed analysis of the interactions between DbpA and decorin by examining the ability of synthetic DbpA-derived peptides to inhibit DbpA-decorin binding *in vitro*. The most effective inhibitors of decorin binding (p4/5 and p8/9) contained the lysine residues (Lys-82 or Lys-163/Lys-170 for p4/5 and p8/9, respectively) previously found to be critical for decorin binding (26). Chemical modification of the lysine residues within these peptides abolished their inhibitory activity, confirming the importance of these residues in DbpA-decorin interactions. Peptides containing Lys-82 (p4/5 region) were significantly better inhibitors than Lys163/Lys170-containing peptides (p8, p9, and p8/9). Furthermore, a peptide composed of a scrambled 4/5A sequence had no inhibitory activity, whereas a scrambled 8/9 sequence retained almost all of the inhibitory activity. Also, an antibody to the 4/5 peptide partially inhibited DbpA binding to decorin, whereas anti-8/9 serum had no significant inhibitory effects. Taken together, these observations suggested that the 4/5 sequence represents a decorin-binding site in DbpA, whereas the 8/9 sequence and the Lys-163 and Lys-170 residues are important in maintaining the ligand binding activity in DbpA, perhaps by maintaining the appropriate decorin-binding conformation.

The shortest peptide from the 4/5 region with inhibitory properties was a 15 amino acid-long peptide (peptide 4/5C) with the sequence PFILEAKVRATTVAE. Further truncations of this peptide (4–5 amino acids) resulted in inactive peptides. Previously, CD spectroscopy of the decorin-binding proteins revealed that they had a high content (~50–60%) of  $\alpha$ -helices (17). CD spectroscopy of peptides p4/5, p4/5A, p4/5C, p4/5D, and p8/9 was also performed, and deconvolution of the spectra revealed that the peptides under the conditions used (1% PBS)



contained both coiled and  $\beta$ -sheet structural elements (data not shown).

DbpA peptides from five distinct *Borrelia* strains, ranging from 27 to 93% in identity with peptide 4/5C from strain 297, all showed significant decorin inhibitory activity, indicating that this is the major decorin-binding site of DbpA regardless of the origin of the MSCRAMM. These peptides represent all of the three subspecies of *Borrelia* known to cause Lyme disease in humans, including the Euro-Asian variants *B. garinii* and *B. afzelii*, which give rise to somewhat different clinical symptoms than infections caused by *B. burgdorferi* sensu stricto strains (34–36). Comparison of DbpA and DbpB amino acid sequences from different *Borrelia* subspecies spanning the p4/5C region showed that while some sequences were identical, others varied (Fig. 9). Phylogenetic analysis of these p4/5C sequences indicated that all of the DbpA sequences from the different subspecies were more closely related to each other than to the DbpB sequences. Sequences within the DbpA group clustered according to the subspecies with only a few exceptions (data not shown). Similar results were obtained when the analysis was performed using the entire DbpA sequences. This suggested that *Borrelia* acquired these genes in the relatively early stage of evolution so that significant divergence appeared in sequences from different subspecies, whereas the sequences within the subspecies were relatively conserved. Multiple sequence alignment indicated that residues A and K were conserved in all of the sequences including the DbpB sequences, indicating that these residues are critical for decorin binding. Based on the multiple sequence alignment of the different p4/5C sequences of both DbpA and DbpB in Fig. 9, a consensus sequence was built using a threshold of 80% identical: h-I-h-c-A-K-l-p-u-h-p-l-s-t (h, hydrophobic; c, charged; l, (I, L, or V); u, (A, G, or S); p, polar; s, small; and t, turn-like). A more defined consensus was built using a threshold of 50% identical: F-I-l-X-A-K-l-R-A-s-s-V-A-E. Based on this, the sequence FILXA-KIRAXXVAE (X, any amino acid residue) was used to blast-search the databases of the human, mouse, and microbial genomes at NCBI. Sequences that contained the conserved Ala and Lys residues and fit the profile of the first consensus were found in a number of microorganisms as well as in human and mouse. Some of the blast hits revealed proteins of known functions or of intracellular proteins, suggesting these hits could be random. However, several hits of potential interest were found. Two hypothetical proteins from *Bacillus anthracis* and *Chlamydia trachomatis*, respectively, contained portions of the decorin binding motif and were of similar length to DbpA. The *B. anthracis* protein had 172 amino acid residues, and the motif was present from residues 139 to 154 (FILDMTA-KIRAMGLTA) with two extra residues (Asp and Met) inserted between Leu and Thr. The *C. trachomatis* protein contained 153 amino acid residues, and a part of the motif was found from residues 98 to 105 (FIIDAKIR). The primary amino acid sequences of these two proteins were not significantly similar to those of DbpA or DbpB. The finding of a putative decorin binding motif in these sequences may suggest a biological function for these proteins. A similar motif was also identified in human tuftelin 1 sequence from residues 245 to 258 (AL-LAKVREGGEVALE). Tuftelin was proposed to play a role in tooth development and the mineralization of enamel; however, the expression of tuftelin genes was not limited to mineralizing tissues, suggesting that the protein may have other functions (37). It will be of interest to see whether any of these proteins can in fact bind decorin and if so what the biological consequences are.

It is also likely that DbpB has a decorin-binding site that includes the conserved Lys-82 residue considering the similarity between DbpA and DbpB in this region and the fact that

DbpA and DbpB share binding sites in decorin. Although no three-dimensional structures of the Dbps are yet available, circular dichroism spectra of recombinant DbpA, DbpB, and the outer surface protein C are very similar (17), indicating similar secondary structures. Outer surface protein C was shown by x-ray crystallography to be a largely  $\alpha$ -helical protein with two short  $\beta$ -sheets (38). Information on the three-dimensional structure of DbpA or DbpB, in combination with our data, would more clearly define the decorin-binding site of these adhesins.

## REFERENCES

- Steere, A. C., Green, J., Schoen, R. T., Taylor, E., Hutchinson, G. J., Rahn, D. W., and Malawista, S. E. (1985) *N. Engl. J. Med.* **312**, 869–874
- Steere, A. C. (1989) *N. Engl. J. Med.* **321**, 586–596
- Steere, A. C., Sikand, V. K., Meurice, F., Parenti, D. L., Fikrig, E., Schoen, R. T., Nowakowski, J., Schmid, C. H., Laukamp, S., Buscarino, C., and Krause, D. S. (1998) *N. Engl. J. Med.* **339**, 209–215
- Steere, A. C., Levin, R. E., Molloy, P. J., Kalish, R. A., Abraham, J. H., III, Liu, N. Y., and Schmid, C. H. (1994) *Arthritis Rheum.* **37**, 878–888
- Dattwyler, R. J., and Halperin, J. J. (1987) *Arthritis Rheum.* **30**, 448–450
- Trollmo, C., Meyer, A. L., Steere, A. C., Hafner, D. A., and Huber, B. T. (2001) *J. Immunol.* **166**, 5286–5291
- Gross, D. M., Forsthuber, T., Tary-Lehmann, M., Etling, C., Ito, K., Nagy, Z. A., Field, J. A., Steere, A. C., and Hubber, B. T. (1998) *Science* **281**, 703–706
- Fraser, C. M., Norris, S. J., Weinstock, G. M., White, O., Sutton, G. G., Dodson, R., Gwin, M., Hickey, E. K., Clayton, R., Ketchum, K. A., Sodergren, E., Hardham, J. M., McLeod, M. P., Salzberg, S., Peterson, J., Khalak, H., Richardson, D., Howell, J. K., Chidambaram, M., Utterback, T., McDonald, L., Artiach, P., Bowman, C., Cotton, M., Fujii, C., Garland, S., Hatch, B., Horst, K., Roberts, K., Sandusky, M., Weidman, J., Smith, H. O., and Venter, J. C. (1998) *Science* **281**, 375–388
- Casjens, S. (2000) *J. Mol. Microbiol. Biotechnol.* **2**, 401–410
- Hellwege, J., Meri, T., Heikkilä, T., Alitalo, A., Panelius, J., Lahdenne, P., Seppälä, I. J., and Meri, S. (2001) *J. Biol. Chem.* **276**, 8427–8435
- Coburn, J., Barthold, S. W., and Leong, J. M. (1994) *Infect. Immun.* **62**, 5559–5567
- Coburn, J., Leong, J. M., and Erban, J. K. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 7059–7063
- Coburn, J., Chege, W., Magoun, L., Bodary, S. C., and Leong, J. M. (1999) *Mol. Microbiol.* **34**, 926–940
- Guo, B. P., Norris, S. J., Rosenberg, L. C., and Höök, M. (1995) *Infect. Immun.* **63**, 3467–3472
- Parveen, N., and Leong, J. M. (2000) *Mol. Microbiol.* **35**, 1220–1234
- Probert, W. S., and Johnson, B. J. (1998) *Mol. Microbiol.* **30**, 1003–1015
- Guo, B. P., Brown, E. L., Dorward, D. W., Rosenberg, L. C., and Höök, M. (1998) *Mol. Microbiol.* **30**, 711–723
- Roberts, W. C., Mullikin, B. A., Lathigra, R., and Hanson, M. S. (1998) *Infect. Immun.* **66**, 5275–5285
- Hocking, A. M., Shinomura, T., and McQuillan, D. J. (1998) *Matrix Biol.* **17**, 1–19
- Iozzo, R. V., Moscatello, D. K., McQuillan, D. J., and Eichstetter, I. (1999) *J. Biol. Chem.* **274**, 4489–4492
- Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V. (1997) *J. Cell Biol.* **136**, 729–743
- Cheng, F., Heinegard, D., Malmstrom, A., Schmidtchen, A., Yoshida, K., and Fransson, L. A. (1994) *Glycobiology* **4**, 685–696
- Iozzo, R. V. (1998) *Annu. Rev. Biochem.* **67**, 609–652
- Henry, S. P., Takanosu, M., Boyd, T. C., Mayne, P. M., Eberspaecher, H., Zhou, W., de Crombrughe, B., Hook, M., and Mayne, R. (2001) *J. Biol. Chem.* **276**, 12212–12221
- Lorenzo, P., Asperg, A., Onnerfjord, P., Bayliss, M. T., Neame, P. J., and Heinegard, D. (2001) *J. Biol. Chem.* **276**, 12201–12211
- Brown, E. L., Guo, B. P., O'Neal, P., and Höök, M. (1999) *J. Biol. Chem.* **274**, 26272–26278
- Choi, H. U., Johnson, T. L., Pal, S., Tang, L. H., Rosenberg, L. C., and Neame, P. J. (1989) *J. Biol. Chem.* **264**, 2876–2884
- Patti, J. M., House-Pompeo, K., Boles, J. O., Garza, N., Gurusiddappa, S., and Höök, M. (1995) *J. Biol. Chem.* **270**, 12005–12011
- Brown, E. L., Wooten, R. M., Johnson, B. J., Iozzo, R. V., Smith, A., Dolan, M. C., Guo, B. P., Weis, J. J., and Höök, M. (2001) *J. Clin. Invest.* **107**, 845–852
- Weis, J. J. (2002) *Curr. Opin. Rheumatol.* **14**, 399–403
- Revel, A. T., Talaat, A. M., and Norgard, M. V. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 1562–1567
- Liang, F. T., Nelson, F. K., and Fikrig, E. (2002) *J. Exp. Med.* **196**, 275–280
- Carroll, J. A., Cordova, R. M., and Garon, C. F. (2000) *Infect. Immun.* **68**, 6677–6684
- Ornstein, K., Berglund, J., Bergstrom, S., Norrby, R., and Barbour, A. G. (2002) *Scand. J. Infect. Dis.* **34**, 341–346
- Ishii, N., Isogai, E., Isogai, H., Kimura, K., Nishikawa, T., Fujii, N., and Nakajima, H. (1995) *Microbiol. Immunol.* **39**, 929–935
- Hubalek, Z., and Halouzka, J. (1997) *Eur. J. Epidemiol.* **13**, 951–957
- Deusch, D., Leiser, Y., Shay, B., Feron, E., Taylor, A., Rosenfeld, E., Dafni, L., Charuvi, K., Cohen, Y., Haze, A., Fuks, A., and Mao, Z. (2002) *Connect. Tissue Res.* **43**, 425–434
- Eicken, C., Sharma, V., Klabunde, T., Owens, R. T., Pikas, D. S., Höök, M., and Sacchetti, J. C. (2001) *J. Biol. Chem.* **276**, 10010–10015