The Complement Regulator Factor H Binds to the Surface Protein OspE of Borrelia burgdorferi*

Received for publication, August 31, 2000, and in revised form, November 30, 2000
Published, JBC Papers in Press, December 11, 2000, DOI 10.1074/jbc.M007994200

Jens Hellwage‡‡, Taru Meri‡‡, Tero Heikkilä‡‡, Antti Alitalo‡‡, Jaana Panelius‡‡, Pekka Lahdenne‡‡, Ilkka J. T. Seppälä‡‡, and Seppo Meri‡‡**

From the ‡Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Haartmaninkatu 3, FIN-00014 Helsinki, Finland, the §Hospital for Children and Adolescents and ¶Molecular Immunodiagnostic Group, Hans Knoell Institute for Natural Products Research, Beutenbergstrasse 11a, D-07745 Jena, Germany

Spirochete bacteria of the Borrelia burgdorferi sensu lato complex cause Lyme borreliosis. The three pathogenic subspecies Borrelia garinii, Borrelia afzelii, and Borrelia burgdorferi sensu stricto differ in their disease profiles and susceptibility to complement lysis. We investigated whether complement resistance of Borreliae could be due to acquisition of the main soluble inhibitors of the alternative complement pathway, factor H and the factor H-like protein 1. When exposed to nonimmune EDTA-plasma, the serum-resistant B. afzelii and B. burgdorferi sensu stricto strains bound factor H/factor H-like protein 1 to their surfaces. Assays with radiolabeled proteins showed that factor H bound strongly to the B. burgdorferi sensu stricto strain. To identify factor H ligands on the borrelian surface, we analyzed a panel of outer surface proteins of B. burgdorferi sensu stricto with the surface plasmon resonance technique. The outer surface lipoprotein OspE was identified as a specific ligand for factor H. Using recombinant constructs of factor H, the binding site for OspE was localized to the C-terminal short consensus repeat domains 15–20. Specific binding of factor H to B. burgdorferi sensu stricto OspE may help the pathogen to evade complement attack and phagocytosis.

* This work was supported by the Life 2000 Program of the Academy of Finland, the Sigrid Juselius Foundation, the Foundation for Pediatric Research, the Helsinki University Central Hospital Funds, the National Technology Agency of Finland, the University of Helsinki, and by a postdoctoral fellowship from the Hochschulsunderprogramm III of the Deutscher Akademischer Austauschdienst (to J. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Dept. of Bacteriology and Immunology, Haartman Inst., University of Helsinki, Haartmaninkatu 3, FIN-00014 Helsinki, Finland. Tel.: 358-9-1912-6758; Fax: 358-9-1912-6382; E-mail: meri@helsinki.fi.

The complement (C)1 system is an important effector system of the innate immunity in the first line of defense against invading microbes. C activation is a major mechanism whereby the spirochetes become coated by opsonizing molecules (C1q, C3b, iC3b) after entering the human host. This may be initiated by antibodies or antibody-independently via direct activation of the classical or the alternative C pathway. Activation of the C membrane attack complex usually leads to lysis of Gram-negative bacteria, unless the microbes have a means to escape the attack.

Several factors may be involved in the interplay between Borrelia bacteria and their different hosts. Recent studies support the importance of the C system before and during the infection. The tick Ixodes scapularis expresses a complement-regulatory protein in its saliva, which down-regulates C activation at an early stage in the midgut of the tick (16). The spirochetes may benefit from the protective effect of this protein, first, after the tick’s blood meal when the spirochetes are still in the tick but already in contact with human plasma, and second, when the pathogens in the tick saliva enter the human body. However, at least after the transmission of the pathogen of the genus Ixodes, which transmit the bacteria between the different mammals. The prolonged survival indicates that the spirochetes are capable of effectively evading the host immune mechanisms. During the different stages of its life cycle, Borrelia shows dramatic changes in the expression of its surface proteins (5, 6). It has been shown that many of the of borrelian surface proteins raise an antibody response in the human host and in animal models (7–9). However, only a few of the antigens have proven useful as vaccine candidates. Recently developed Lyme disease vaccines use either the outer surface protein A (OspA) (10, 11) or OspC (12) as an immunogen. OspA is mainly expressed while the spirochetes persist in the tick and the expression of OspA is already down-regulated when the pathogen is transmitted to the human host. The OspA vaccine leads to a 70–90% protection (10, 11, 13). On the other hand, OspC is preferentially expressed in the mammalian host. In an optimal situation, antibodies against vaccine proteins would neutralize important virulence factors on the surface of the spirochetes. The physiological roles of most of the borrelian antigens are yet unknown. However, the recently deciphered genome sequence of B. burgdorferi sensu stricto serves as a basis also for functional studies (14, 15).

1 The abbreviations used are: C, complement; AP, alternative pathway of complement; FHL-1, factor H-like protein 1; SCR, short consensus repeat domain; VBS, veronal-buffered saline; GVB, veronal-buffered saline with gelatin; FHR, factor H-related protein; NHS, normal human serum; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase.
to the human host and dissemination of Borreliae from the site of infection, the spirochetes must cope with the powerful effects of the human C system. The three main subspecies of the B. burgdorferi sensu lato complex have been tested for their sensitivity to serum complement-mediated bacteriolysis. Although B. garinii strains were considered serum-sensitive, the B. afzelii and B. burgdorferi sensu stricto strains were resistant or partially resistant with no effective deposition of the terminal complement complexes on the pathogen surface (17, 18). Analogously, differences in complement-mediated opsonophagocytosis of the different Borrelia subspecies have been observed. In an assay dependent on an interaction between iC3b and the integrin CD11b/CD18 (CR3, Mac-1), neutrophils showed a strong response to serum-opsinized B. burgdorferi sensu stricto (19).

An important mechanism of complement activation is the alternative pathway (AP). The AP is unique in its spontaneous initiation and the random nature of C3b deposition on target structures. The latter leads to an attack against all particles, membranes, and cells that are not specifically protected against AP activation (20). In addition to direct activation, the AP can enhance C activation initiated by the classical or the lectin pathway. Complement activation via AP is strictly controlled by a number of membrane-bound proteins on host cell surfaces and by the fluid phase regulators factor H and its truncated form factor H-like protein 1 (FHL-1) (21, 22). Factor H is composed of 20 and FHL-1 of seven short consensus repeat (SCR) domains. Factor H and FHL-1 control AP activation in three ways. They (i) act as cofactors for the serine protease factor I in the cleavage of C3b; (ii) accelerate the decay of the AP C3 convertase, C3bBb, which promotes the amplification of complement activation by cleaving C3 into its active form C3b; and (iii) compete with factor B for binding to C3b and thus prevent formation of the AP C3 convertase (23–26). Recently, increasing interest has been focused on the interactions between microbial surface proteins and human complement regulators. Exploitation of C inhibitors of the host could be an attractive strategy to prevent formation of the AP C3 convertase (23–26). Recently, the binding site of factor H for OspE was located on the outer surface protein E (OspE) as a specific ligand for factor H (27). OspE binds to different subspecies of the B. burgdorferi sensu stricto, promoting killing of Borrelia in human serum.

The binding site of factor H for OspE was located on the C-terminal part of the molecule. Binding of factor H to OspE may help Borreliae to evade C-mediated opsonophagocytosis and direct killing.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Recombinant Borrelia Surface Proteins**—The recombinant OspE protein was generated by PCR amplification of the OspE gene of the American B. burgdorferi sensu stricto strain N40 (39). The OspE gene was cloned into a pGEX-2T vector and expressed in BL21 (DE3) (La Jolla, CA). Recombinant proteins FHL-1 (representing the N-terminal seven SCRs of factor H plus four unique additional amino acids), SCRs 8–20 and SCR 15–20 of factor H, factor H-related proteins FHR-3 and FHR-4, and a construct SCR 1–4 of FHR-3 were expressed with the baculovirus expression system as described previously (31–34). The recombinant proteins were purified by Ni2+-charged chromatography as described (35). Purity of the proteins was analyzed by SDS-PAGE and silver staining.

**Bacterial Strains**—Borrelial strains B. burgdorferi sensu stricto (Bbia), isolated from the cerebrospinal fluid of a Finnish neuroborreliosis patient, B. afzelii (BaA91, 1052) and B. garinii (Bgl3, -28, -40, -46, and -50), isolated from skin biopsies of Finnish patients with erythema migrans, were kindly provided by Matti Viljanen (National Public Health Institute, Turku, Finland). The B. afzelii strain 570 was isolated from a tick in the Helsinki park area. The strains were identified by the biotyping procedure (38) and the PCR amplification of the flagellin gene of B. burgdorferi (36). Briefly, a 277-base pair segment of the flagellin gene was obtained by PCR using primers FL7 (biotinylated) and FL59 (37). The biotinylated PCR products were rendered single-stranded using streptavidin-coated Dynabeads according to the instructions of the manufacturer. The Dynabeads M-280 streptavidin; Dynal AS, a saline wash, and sonication. The GST-OspE fusion protein was expressed in Escherichia coli DH5-α host cells. Briefly, a primary culture was started by inoculating a single colony from a fresh transformant plate to 50 ml of Luria-Bertani (LB) broth containing 100 µg/ml ampicillin. The culture was incubated at 37 °C with shaking overnight. The culture was diluted 1:50 to 1500 ml of LB broth containing 100 µg/ml ampicillin and incubated at 37 °C for 3 h (until the growth reached the mid-log phase with an optical density of ~0.6 at 600 nm). Isopropyl-β-D-thiogalactoside was added to a final concentration of 0.7 mM. After an additional incubation of 3 h, the cells were harvested, washed, and sonicated. The GST-OspE fusion protein was purified by affinity chromatography on glutathione-Sepharose matrix according to the instructions of the manufacturer (Amersham Pharmacia Biotech). The recombinant GST-OspE was cleaved from GST with bovine thrombin (Sigma) in thrombin cleavage buffer (150 mM NaCl, 100 mM KCl, 2.5 mM CaCl2, 1 mM diithiothreitol, 20 mM Hepes, pH 7.6) for 3 h and then eluted with thrombin cleavage buffer. The cleaved GST-OspE was dialyzed against 10 µl, pH 8.6 for 20 h. The dialyzed GST-OspE was applied on a MonoQ (Amersham Pharmacia Biotech) anion exchange column in the same buffer and eluted by adding a gradient of NaCl to the starting buffer. The purity of the OspE protein after MonoQ purification was confirmed by SDS-PAGE.

OspA, OspD, and decorin-binding protein A (DbpA) were from the American B. burgdorferi sensu stricto strains 297, N40, and N40, respectively. They were cloned originally in Michael Norgard’s laboratory (Oslo, Norway). Manual sequencing was performed by using Sanger’s dideoxynucleotide chain termination method and Sequenase 2.0 (U. S. Biochemical Corp., Cleveland, OH) as described previously (38). The obtained sequences were compared with the flagellin gene sequences of the type strains of B. afzelii Bo23 and B. garinii 387. All isolates were grown in BSK-H medium (Sigma) at 33 °C in 5% CO2 atmosphere until the cultures reached the late exponential phase. The cultures were harvested by centrifugation, washed, and diluted to a final bacterium concentration of 2 × 107/ml in veronal-buffered saline (VBS) or 1/3 GVB (1/3× VBS with 0.1% gelatin), and subsequently used in complement component binding experiments.

**Expression and Purification of Recombinant Borrelia Surface Proteins**—The recombinant OspE protein was generated by PCR amplification of the OspE gene of the American B. burgdorferi sensu stricto strain N40 (39). The OspE gene was cloned into a pGEX-2T vector and expressed in E. coli XL1Blue host cells. Their expression and purification were done as described for OspE. The recombinant DbpA was a hexa-His fusion protein construct expressed in E. coli DHS-α host cells, and expression was done as with OspE. DbpA, OspC, and P35/BKK23 recombinant proteins were generated by PCR amplification of the DNA encoding the predicted mature portion of these proteins from the Finnish B. burgdorferi sensu stricto strain 570. All isolates were grown in BSK-H medium (Sigma) at 33 °C in 5% CO2 atmosphere until the cultures reached the late exponential phase. The cultures were harvested by centrifugation, washed, and diluted to a final bacterium concentration of 2 × 107/ml in veronal-buffered saline (VBS) or 1/3 GVB (1/3× VBS with 0.1% gelatin), and subsequently used in complement component binding experiments.
supernatant with the pellet. After cutting off the pellets, the binding of the factor H/FHL-1 proteins was calculated as a percentage of the total radioactivity input. All experiments were performed at least in duplicate and repeated twice. For the heparin inhibition assay, the indicated amounts of low molecular weight heparin (Sigma) were added to the reaction mixture simultaneously with factor H/FHL-1.

The inhibitory effects of increasing amounts of OspE (ranging from 0 to 300 µg/ml) or 300 µg of OspA (control protein) on the binding of factor H to whole B. burgdorferi sensu stricto was assayed similarly by adding the competing proteins together with 125I-labeled factor H.

Effect of Soluble OspE on the Survival of B. burgdorferi in Human Serum—The B. burgdorferi sensu stricto (strain ia), B. afzelii (strain 1082), and B. garinii (strain 46) were cultured as described above. Freshly harvested or frozen bacteria (6.4 × 10^9) were washed with GVB and incubated with serum for 2 h at 37 °C in heat-inactivated serum (control); NHS (nonimmune serum, no IgG or IgM antibodies against B. burgdorferi detectable by enzyme-linked immunosorbent assay; Ref. 40); or NHS supplemented with 0, 20, or 200 µg/ml (final concentration) of soluble OspE protein. Serum was added after 30 and 60 min to avoid depletion of complement components caused by blocking factor H. The final serum concentration shifted from 20% to 33% during the experiment. The surviving bacteria were counted microscopically after 2 h, and the percentage of live spirochetes was calculated.

Immunofluorescence Analysis of C3 and Factor H Binding to Borrelia Spirochetes—B. burgdorferi sensu stricto (strain ia), B. afzelii (strain 1082), and B. garinii (strain 46) were cultured as described above. Freshly harvested sensu stricto strain (6.4 × 10^9) or frozen bacteria (B. garinii (5.4 × 10^9) and B. afzelii (6.4 × 10^9)) were washed with GVB and incubated 1:2 with nonimmune NHS or EDTA-plasma from the same donor for 60 min at 37 °C. Bacteria were washed three times with GVB and incubated with a monoclonal antibody against the ic3b neoepitope (Quidel Corp., La Jolla, CA). For detection of factor H, the monoclonal antibody VIG8 (kindly provided by Dr. Wolfgang Prodinger, University of Innsbruck, Austria; Refs. 32 and 41) for specific detection of the C terminus (SCRs 19–20) of factor H was used. An unrelated B-cell lymphoma idiotype-specific AF1 antibody served as a control. Samples were incubated for 30 min at 37 °C with primary antibodies (10 µg/ml) and washed three times with GVB before adding a polyclonal fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Alexa 488, Molecular Probes, Eugene, OR) used at a 1:200 dilution. The stained samples were mounted with Mowiol (42) and examined on an Olympus BX50.

Surface Plasmon Resonance Assays—Protein-protein interactions were analyzed in real time by the surface plasmon resonance technique using the Biacore 2000 instrument as described recently (33, 34). B. burgdorferi sensu stricto (strain ia) proteins OspA, OspC, OspD, OspE, P35/BBK32, and DbpA were immobilized via a standard amine-coupling procedure to flow cells of a sensor chip (carboxylated dextran chip CM5; Biacore AB, Upsala, Sweden). Two flow cells were activated with 35 µl of a mixture of 0.2 M N-ethyl-N’-(di-methylamino)propylcarbodiimide and 0.05 M N-hydroxysuccinimide (Biacore AB). The protein to be immobilized was dialyzed against 10 mM acetate buffer (pH 4.8–5.5) and 20 µg portions (~150 µg/ml) of the proteins were injected into one of the flow cells until an appropriate level of coupling for the binding experiments (~4,000 resonance units) was reached. The other flow cell without any protein was used as a control. Unreacted groups in both flow cells were inactivated by a standard ethanoldine-HCl injection (35 µl). After the coupling procedure, the flow cells were washed thoroughly with sequential injection of 1/3 VBS, pH 7.4, and 3 mM NaCl in 10 mM acetate buffer, pH 4.6.

First, the binding of a panel of Borrelia surface proteins (OspA, OspC, OspD, OspE, P35/BBK32, DbpA) to factor H was tested. The proteins were dialyzed against 1/3 VBS, and protein concentrations were measured using the BCA protein assay (Pierce). Each ligand was injected separately to a blank control flow cell and the flow cell with factor H using a flow rate of 5 µl/min at 22 °C. The final concentrations of the proteins in the binding assay ranged from 0.25 to 200 µg/ml. In a second set of experiments, a reverse setting was used. The Borrelia surface proteins were immobilized to the sensor chip surface as described above. Factor H, recombinant deletion constructs of factor H, or factor H-related proteins (proteins which are similar but not identical to factor H; Ref. 43) were injected to a flow cell coated with a borrelial surface protein and to a blank channel. Binding was assayed at least in duplicate using independently prepared sensor chips.

**Fig. 1.** Immunofluorescence analysis of serum ic3b deposition and factor H/FHL-1 binding to different strains of B. burgdorferi. The strains B. burgdorferi sensu stricto (strain ia), B. afzelii (strain 1082, and B. garinii 46 were treated with undiluted nonimmune NHS or EDTA-plasma (30 min, 37 °C), washed, and immunostained with specific monoclonal antibodies against ic3b (row 1) or factor H. The antibody VIG8 (rows 2 and 3) detects the C-terminal SCR 19–20 of domain of factor H. In the control (row 4), the serum-treated bacteria were incubated with an unrelated antibody (AF-1) against an idiotypic B cell surface marker. Bound antibodies were detected with a fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody. Controls were negative for all strains.

**RESULTS**

**Binding of Factor H and FHL-1 to Different Strains of B. burgdorferi**—The binding of human complement regulatory proteins as one mechanism to evade complement attack and phagocytosis has been reported recently for some pathogenic organisms, like Streptococcus pyogenes (44–46), Streptococcus pneumoniae (47), and Neisseria gonorrhoeae (48, 49). Our first aim was to investigate whether the 150-kDa factor H protein and/or its truncated 42-kDa form FHL-1, the main soluble inhibitors of the alternative pathway of complement, bind to the surfaces of the spirochete bacteria of the B. burgdorferi sensu latu complex.

**Immunofluorescence Microscopy Analysis of C3 and Factor H Binding to Borrelia**—To examine complement deposition and regulation on the borrelial surface, we incubated spirochetes of the three subspecies with nonimmune human serum or EDTA plasma. Complement C3 activation and inactivation by ic3b and factor H binding were analyzed by immunofluorescence microscopy using specific antibodies (Fig. 1). With an antibody directed against an ic3b neoepitope, strong complement deposition was detected on all the three strains. This indicated that incubation of Borrelia in NHS led to complement activation on the borrelial surface. An antibody specific for the C-terminal domain SCR 19–20 of factor H (VIG8) showed strong signals for the B. burgdorferi sensu stricto and for B. afzelii strains but
specifically with a ligand on the surface of the bacteria (data not shown). Factor H and FHL-1 thus seem to interact with the borrelial surface, the experiment was re-run with the strains (Fig. 2). To test the specificity of this interaction for the borrelial surface, the experiment was repeated with the strains (Fig. 2). The values were 10 times higher for factor H and 5 times higher for FHL-1 compared with the control strain. Factor H from B. garinii and B. burgdorferi sensu stricto/ia strain was weaker. A noncomplement antibody, serving as a negative control, showed no positive staining at all (Fig. 1, last row).

With the next experiment we wanted to analyze direct binding of factor H and FHL-1 to Borrelia in the absence of any possibly interfering serum component. Radiolabeled factor H and FHL-1 proteins were incubated (30 min at 37 °C) with six different strains of the three subspecies of B. burgdorferi: B. garinii strains 13, 28, and 50; B. afzelii 570 and 1082; and B. burgdorferi sensu stricto strain ia. The deposition on B. garinii was weaker. A noncomplement antibody, serving as a negative control, showed no positive staining at all (Fig. 1, last row).

a weaker staining for the B. garinii strain. Factor H from serum may bind to C3b deposited on the borrelial surface. In addition, the binding might have been enhanced by a specific ligand on the spirochete. After incubation of Borrelia in NHS-EDTA, where complement activation is inhibited, factor H binding was detected only to the serum-resistant B. burgdorferi sensu stricto and B. afzelii strains. The deposition on B. garinii was weaker. A noncomplement antibody, serving as a negative control, showed no positive staining at all (Fig. 1, last row).

With the next experiment we wanted to analyze direct binding of factor H and FHL-1 to Borrelia in the absence of any possibly interfering serum component. Radiolabeled factor H and FHL-1 proteins were incubated (30 min at 37 °C) with six different strains of the three subspecies of B. burgdorferi: B. garinii strains 13, 28, and 50; B. afzelii 570 and 1082; and B. burgdorferi sensu stricto strain ia (each at 2 × 10⁷ cells ml⁻¹). The bound protein was separated by centrifugation through a 20% sucrose solution. Radioactivity was quantified with a γ counter. A parallel batch without addition of bacteria served as a negative control (control).

With the next experiment we wanted to analyze direct binding of factor H and FHL-1 to Borrelia in the absence of any possibly interfering serum component. Radiolabeled factor H and FHL-1 proteins were incubated (30 min at 37 °C) with six different strains of the three subspecies of B. burgdorferi: B. garinii strains 13, 28, and 50; B. afzelii 570 and 1082; and B. burgdorferi sensu stricto strain ia (each at 2 × 10⁷ cells ml⁻¹). The bound protein was separated by centrifugation through a 20% sucrose solution. Radioactivity was quantified with a γ counter. A parallel batch without addition of bacteria served as a negative control (control).

Influence of Soluble OspE on the Interaction between Factor H and Borrelia—To test the specificity of factor H interaction with the surface protein OspE, we measured the effect of soluble OspE on the binding of factor H to Borrelia. As shown in Fig. 5, OspE derived from B. burgdorferi sensu stricto inhibited the binding of factor H in a dose-dependent manner. At a concentration of 300 μg/ml OspE, the binding was completely abrogated. The protein OspA, used as a control, did not show a significant effect. OspE thus seems to be a specific target structure for factor H on the surface of B. burgdorferi sensu stricto.

Binding of OspE to Other Complement Components—To further assess the specificity of factor H binding to OspE, we examined binding of OspE to two other complement components, C3b and C5. For this assay, the binding of C3b or C5 in the fluid phase or the reverse setting with immobilized C3b or C5 and soluble recombinant OspE and P35/BBK32 proteins was used. As shown in Fig. 6, no direct interaction between C3b or C5 and OspE was observed. The reverse setting with immobilized C3b or C5 and soluble-phase OspE showed the same negative results. In contrast, however, the P35/BBK32 protein bound to both C3b and C5, when the latter proteins were coupled to the chip (data not shown). Thus, the interaction between P35/BBK32 and OspE was observed.
BBK32 and factor H/C3b/C5 seems to be more due to a general “stickiness” of the protein rather than to a specific interaction. In conclusion, from the panel of surface proteins that were tested, OspE was identified as a specific ligand for the complement regulator factor H.

**Effect of Soluble OspE Protein on the Survival of Different B. burgdorferi**

**FIG. 4.** Analysis of *Borrelia* surface proteins for binding of factor H by the surface plasmon resonance technique. Binding of proteins to the immobilized ligand is detected as an increase of resonance units per time. Factor H in the fluid phase (125 µg/ml in 1/3 VBS, 10 µl) was injected into a flow cell precoupled with borrelial surface proteins (solid lines) and into the control flow cell (blank channel, plotted as dotted lines), where only the bulk effect is seen. The left part of the panel shows the binding results for OspA (A), OspD (B), and DbpA (C) to immobilized factor H. The right part shows binding analysis of factor H to immobilized OspC (D), P35/BBK32 (E), and OspE (F). All recombinant fragments were tested on two different chip surfaces, and representative figures are shown in panels A–F. Note the strong binding of factor H to OspE, but not to the other *Borrelia* surface proteins.

**FIG. 5.** Inhibition of interaction between factor H and *Borrelia* by soluble OspE protein. Binding of factor H to the borrelial surface was measured as described for Fig. 2. The influence of increasing amounts of OspE (0–300 µg/ml) or OspA (control protein) on the binding of factor H to whole *B. burgdorferi* sensu stricto was assayed.

**FIG. 6.** Binding of complement components C3b and C5 to OspE. The ability of OspE to bind to the complement components C3b and C5 was tested using a biosensor assay similarly as described for Fig. 4. OspE was immobilized on the chip surface, and C3b and C5 were injected as fluid-phase ligands. In the control flow cell, no protein was immobilized.
B. burgdorferi Interaction with Complement Factor H

**FIG. 7. Effect of soluble OspE protein on the survival of different B. burgdorferi strains in human serum.** The indicated strains were incubated in heat-inactivated serum (control), in normal human serum without adding OspE, or in normal human serum supplemented with 20 or 200 μg/ml (final concentrations) of soluble OspE protein. Serum was added stepwise to avoid depletion of complement components. The percentage of surviving bacteria after 2 h was counted microscopically.

**burgdorferi Strains in Human Serum—**The B. burgdorferi sensu stricto, B. burgdorferi afzelii, and B. burgdorferi garinii strains were incubated either in heat-inactivated serum (control), normal human serum without adding OspE or in normal human serum supplemented with 20 or 200 μg/ml soluble OspE (Fig. 7). The mixture was replenished twice with serum to compensate for potential depletion of the AP in the fluid phase because of blockade of the regulator factor H. After 2 h, the number of surviving bacteria was counted microscopically. In normal human serum, the survival rate was reduced to 30% as compared with 90% in heat-inactivated serum. When OspE was added at a concentration of 20 or 200 μg/ml, only 10% or 5% of bacteria survived, respectively. B. burgdorferi afzelii showed a much higher serum resistance and was not affected by the addition of human normal serum. Only at the high OspE concentration the survival rate was reduced from 80% to 70%. The B. burgdorferi garinii strain, known to be serum-sensitive, showed 20% survival in the absence of OspE and only 1% of the spirochetes survived when OspE was added. Thus, OspE seems to be an important factor for the survival of B. burgdorferi in human serum, in particular for the sensu stricto strain.

**Mapping of the OspE Binding Site on Factor H—**As we had identified OspE as a specific ligand for factor H, we wanted to localize the binding site on factor H responsible for the interaction. To this end, we tested a set of recombinant constructs of factor H and factor H-related proteins for binding to OspE. The binding was again analyzed with the Biacore method. OspE was immobilized on the chip surface, and six different recombinant constructs were tested. The recombinant proteins were expressed in the baculovirus system, and their functional activity has been shown recently (e.g. binding to the complement component C3b) (33, 34). We tested the following constructs of factor H: SCR 1–7 (FHL-1), SCR 8–20, and SCR 15–20 (Fig. 8).

Surprisingly, the construct SCR 1–7 did not bind to OspE. This construct contains the domain responsible for the basic complement regulatory functions of factor H in SCRs 1–4 and a domain that has been shown to interact with heparin, streptococcal M protein, and the C-reactive protein. A construct consisting of the SCRs 8–20 clearly bound to OspE. The construct SCR 15–20 of factor H also bound to OspE, suggesting that the interacting site is located in the C-terminal region of the protein.

To confirm the specificity of these results, we used constructs of two factor H-related (FHR) proteins. The FHR proteins are encoded by distinct genes and are similar but not identical to factor H (43). Both FHR-3 and FHR-4 are composed of five SCR units. We used the proteins as “natural mutants” to compare the binding results with those obtained for factor H. The SCR 2 of FHR-3 is homologous to SCR 7 of factor H, and it contains a binding site both for heparin and streptococcal M protein. This binding domain is not present in FHR-4. Fig. 8 shows the result of the binding analysis. Neither the entire FHR-3 nor FHR-4 nor a construct of SCRs 1–4 of FHR-3 bound to OspE. As the construct 15–20 binds to OspE but the FHRs do not, the interaction seems to be mediated by a region that differs between the SCR 15–20 and the FHRs proteins. Thus, we have localized a domain for interaction with OspE to the SCRs 15–20 of factor H, a site that is distinct from the recently described binding site for M proteins of streptococci.

**Influence of Heparin on the Interaction between Factor H and OspE—**To test whether the binding of factor H to the borrelial surface is dependent on charge, the effect of heparin on the interaction between factor H/FHL-1 and Borrelia was investigated. Radiolabeled factor H and FHL-1 were incubated with whole B. burgdorferi sensu stricto bacteria, and the influence of heparin on the binding was analyzed (Fig. 9). At heparin concentrations below 30 μg/ml, a small enhancing effect on factor H binding to the spirochetes was observed. At higher heparin concentrations, the binding of factor H to Borrelia decreased. In contrast, the binding of FHL-1 showed only a small reduction when the heparin concentration increased. Taken together, the results suggest that the C-terminal heparin/sialic acid binding site of factor H is involved in the interaction with B. burgdorferi and the binding site seems to be located on SCR domains 15–20 of factor H.

**DISCUSSION**

In this study we discovered an interaction between the outer surface protein OspE of the human pathogen B. burgdorferi sensu stricto and factor H, the main regulatory protein of the alternative pathway of complement activation. Additional evidence suggests that this may not be the only interaction between factor H and Borrelia. The consequence of factor H binding is that the pathogen can avoid or suppress ongoing complement activation on its surface by inhibiting C3/C5 convertases and promoting the degradation of C3b. As shown by our experiments, OspE is one surface component of Borrelia that can interfere with the human complement system. OspE binds to the C terminus of factor H, thus leaving the N-terminal regulatory domains of the complement inhibitor free to exert their regulatory activities.

Borrelia spirochetes are able to persist for a long time in the human tissues and in circulation. As the complement system is a powerful effector system of the innate immunity, it is advantageous for pathogens to evade complement activation. The direct binding of complement regulators like factor H or its truncated form FHL-1 has recently been reported for several pathogens (27, 28). The best studied example is the interaction with the M protein of group A streptococci (44–46). Both factor H and FHL-1 bind to certain M proteins. FHL-1 seems to be the preferred ligand of the hypervariable region of M proteins, and the binding of the protein does not inhibit its complement regulatory effects. Recently, it has been proposed that factor H also binds to S. pneumoniae and contributes to their resistance to phagocytosis (47). Interactions of factor H with bacterial surfaces have also been reported to contribute to the serum resistance of further pathogens. Factor H binds to sialylated lipo-oligosaccharides of N. gonorrhoeae by an interaction via SCRs 16–20 (48). In addition, factor H also binds to nonsialylated Neisseria strains by an interaction with porin proteins.
the main outer membrane proteins of the pathogen (49). However, the interacting region on factor H responsible for the interaction could not be localized in the latter example.

*Borrelia* is very active in changing its surface properties. The expression of the outer surface proteins, which are in direct contact with the environment, varies according to the alternating hosts, temperature, culture conditions, and several other factors (50–52). The outer surface proteins are thus candidates for a possible interaction with the immune system of the host. We expressed and tested a set of six borrelial surface proteins for their potential binding to factor H/FHL-1: the outer surface lipoproteins OspA, OspC, OspD, and OspE; the fibronectin-binding protein P35/BBK32; and the decorin-binding protein DbpA. OspA is the best studied protein of *Borrelia*. It is the immunogen in a recently developed Lyme disease vaccine (10, 11). OspA is expressed at a high level during the life stage of the spirochete within the tick, but the expression decreases after the tick feeds on blood. On the contrary, the OspC protein is up-regulated after transmission of the bacteria to the mammalian host. When the temperature shifts from 23 °C in the tick to 35 °C in the mammalian host, the expression of the lipoproteins OspE and OspF and of some other antigens is up-regulated among other antigens, an effect that also occurs with *Borrelia* cultures in vitro (50–52). In light of the current results, this phenomenon is beneficial for the spirochete in its ability to resist complement attack. Like many bacterial virulence factors, the 19.2-kDa lipoprotein OspE is encoded by a circular plasmid and coexpressed with OspF in one operon (39). No functions have yet been assigned to OspE. In this study, we describe that OspE binds the plasma complement inhibitor factor H. The interacting region was localized to the C-terminal part of factor H. Binding of factor H to the pathogen surface with this region leaves the complement regulatory domains, which reside in SCRs 1–4 of factor H, free for an efficient

---

**Fig. 8. Mapping of the binding site for OspE on factor H.** Binding was measured as described in Fig. 4. The binding site for OspE on factor H was localized in a biosensor assay with OspE immobilized on the sensor chip surface and the following recombinant constructs, expressed in the baculovirus system, as fluid-phase ligands: SCR 1–7 (FHL-1) (A), SCR 8–20 (B) and 15–20 (C) of factor H, and the factor H-related proteins FHR-3 (D), SCR 1–4 of FHR-3 (E) and FHR-4 (D). The binding curves are shown as solid lines and controls (no protein immobilized) as dotted lines.

**Fig. 9. Inhibition of the interaction between factor H/FHL-1 and *B. burgdorferi* sensu stricto by heparin.** Binding of factor H and FHL-1 to the borrelial surface was measured as described in Fig. 2. The influence of increasing amounts of heparin (0–300 µg/ml) on the binding was assayed.
control of C3b deposition on the bacterial surface similarly as demonstrated earlier for streptococci (45). Direct evidence for the functional relevance of the factor H-OspE interaction came from serum sensitivity tests. Blocking of serum factor H binding to Borrelia burgdorferi sensu stricto by soluble OspE increased the lytic activity of serum toward the bacteria. Our result suggests that OspE could have a potential as a vaccine. However, in mice vaccinated with OspE, no protective immunity against the spirochetes was achieved (53). The situation may, however, be different in humans, as the complement system is species-specific and it is not known whether murine factor H binds to OspE. If antibodies in humans block the complement inhibitory activity of OspE, they would neutralize an important virulence factor and thereby promote destruction of the pathogen.

We also tested the decorin-binding protein DbpA, which has been seen to raise an antibody response in the human host. Although this protein has adhesive properties and may mediate the adherence of spirochetes to collagen fibers in the tissue (54), no interaction with factor H was observed. Another protein, first called P35 and later BBK32, bound to factor H but also to the control channel of our assay. P35/BBK32 has been described to raise a protective antibody response in mice after vaccination (9). Later, this protein was characterized as a fibronectin-binding protein (55, 56). The importance of P35/BBK32 binding to factor H requires further studies because when P35/BBK32 was immobilized on the sensor chip, it did not bind factor H anymore. As P35/BBK32 bound also to C3b and C5 to some extent, the interactions of P35/BBK32 could just indicate a general adhesive property of this protein.

When pathogens evade complement activation, inhibition of the deposition of C3b on the pathogen surface or a late intervention in preventing the formation of membrane attack complexes are possible mechanisms. It has been reported that Borreliae can prevent efficient deposition of membrane attack complex on their surfaces (15). Thus, it is possible that C activation is controlled at the level of C3. In our immunofluorescence stainings, we found deposition of iC3b after serum inactivation also resulted in a deposition of factor H on the surface of all tested strains. When complement activation in plasma was inhibited by EDTA, binding of factor H was observed mainly to B. burgdorferi sensu stricto and B. afzelii, which are strains known to be complement-resistant. The fact that under plasma-free conditions rat sensu stricto and B. afzelii strain may indicate that other plasma factors are needed for the binding to the B. afzelii strain. The binding of factor H to Borrelia was influenced by heparin. Upon increasing the heparin concentration, factor H first showed an increase and then a decrease in binding, while the binding of FHL-1 was reduced to a lesser extent. The reason for this different effect on factor H and FHL-1 binding could be explained by the presence of at least two binding sites for glycosaminoglycans on the longer factor H molecule of which only one is used for binding to Borrelia. Heparin probably dimerizes factor H at low concentrations, thereby increasing the binding activity, whereas at higher concentrations binding is inhibited. Thus, the effect of heparin on the interaction of factor H with Borrelia seems to indicate both oligomerization of factor H molecules and direct inhibition of factor H binding to Borrelia bacteria.

An interaction of Borrelia OspE with factor H is one possible mechanism for the serum resistance of the sensu stricto strain. However, it has been shown that in most cases more than one factor contributes to the resistance. Although we saw binding of FHL-1 to the whole bacteria, on the basis of our experiments OspE does not seem to be a ligand for this complement regulator.

Since FHL-1, nevertheless, bound to B. burgdorferi sensu stricto (Fig. 2) and the immunofluorescence microscopy experiment (Fig. 1) suggested binding of factor H/FHL-1 also to the B. afzelii strain, it is likely that additional ligands for factor H and FHL-1 exist. A study reported at the recent complement workshop investigated the interaction of Borrelia with factor H and FHL-1 (57). Two still unidentified Borrelia proteins were detected by ligand blotting with molecular masses of ~20/21 and 27.5 kDa. The 20/21-kDa protein may be identical with the OspE protein. In conclusion, we have identified a new putative virulence factor of the human pathogen B. burgdorferi sensu stricto, which binds a human complement regulator to evade complement attack. The results bring up OspE as an important functional protein and a potential vaccine candidate for studies in human beings.

Acknowledgments—We thank Michael Norgard for support in developing recombinant borrelian proteins at Marvo Oksi and Matti Viljanen (University of Turku) for borrelian strains, Eva Kampen for expert technical assistance, and the US Highway 91 for inspiration.

REFERENCES

1. Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E., and Davis, J. P. (1982) Science 216, 1317–1319
2. Steere, A. C., Grodzicki, R. L., Kornblatt, A. N., Craft, J. E., Barbour, A. G., Burgdorfer, W., Schmid, G. P., Johnson, E., and Malawista, S. E. (1983) N. Engl. J. Med. 308, 723–745
3. Nadelman, R. B., and Wormser, G. P. (1998) Lancet 352, 557–565
4. Oschmann, P., Derndorf, W., Hurnig, C., Schafer, C., Wellensiek, H. J., and Pflughaupt, K. W. (1998) Neurology 44, 262–272
5. Batsford, S., Rust, C., and Neubert, U. (1998) J. Infect. Dis. 178, 1667–1683
6. Fikrig, E., Feng, W., Aversa, J., Schoen, R. T., and Flavell, R. A. (1998) J. Infect. Dis. 178, 1198–1201
7. Akin, E., McHugh, G. L., Flavell, R. A., Fikrig, E., and Steere, A. C. (1999) Infect. Immun. 67, 173–181
8. Gutierrez, Fernandez, J., Rodriguez, M., Fernandez, M., Nunez Murillo, F., and Maroeto Vela, M. C. (1997) Microbiol. 91, 165–174
9. Fikrig, E., Barthold, S. W., Sun, W., Feng, W., Telford, S. R., III, and Flavell, R. A. (1997) Immunity 6, 531–539
10. Sigal, L. H., Zahradnik, J. M., Lavin, P., Patella, S. J., Bryant, G., Halsey, R., Hilton, E., Kunkel, M., Laukamp, S., Buscarino, C., and Krause, D. S. (1998) N. Engl. J. Med. 339, 209–215
11. Steere, A. C., Grodzicki, R. L., Kornblatt, A. N., Craft, J. E., Barbour, A. G., Burgdorfer, W., Schmid, G. P., Johnson, E., and Malawista, S. E. (1983) N. Engl. J. Med. 308, 723–745
12. Steere, A. C., Benach, J. L., Grunwaldt, E., and Malawista, S. E. (1998) Science 275, 216–218
13. Thansani, W. T., and Schoen, R. T. (2000) Ann. Intern. Med. 132, 661–668
14. Fikrig, E., Barthold, S. W., Sun, W., Feng, W., Telford, S. R., III, and Flavell, R. A. (1997) Immunity 6, 531–539
15. Sigal, E., McHugh, G. L., Flavell, R. A., Fikrig, E., and Steere, A. C. (1999) Infect. Immun. 67, 173–181
16. Van Dam, A. P., Oei, A., Jaspar, C., Fijen, C., Wileke, B., Spanjaard, L., and Dankert, J. (1997) Infect. Immun. 65, 1232–1238
17. Vansteelandt, J. G., Charlah, R., Mattheu, T. N., and Ribeiro, J. M. (2000) J. Biol. Chem. 275, 18717–18723
18. Breitner-Ruddock, S., Wurzner, R., Schulze, J., and Brade, V. (1997) Med. Microbiol. Immunol. 185, 253–260
19. van Dam, A. P., Oei, A., Jaspar, C., Fijen, C., Wileke, B., Spanjaard, L., and Dankert, J. (1997) Infect. Immun. 65, 1228–1236
20. Van den Abbeele, W., Hartela, H., Pflughaupt, K. W., and Villainen, M. K. (1995) J. Infect. Dis. 171, 257–263
21. Pangburn, M. K. (1998) Methods Enzymol. 162, 639–653
22. Liszewski, M. K., Farries, T. C., Lublin, D. M., Rooney, I. A., and Atkinson, J. P. (1996) Adv. Immunol. 61, 201–283
23. Zipfel, P. F., and Skerka, C. (1999) Immuno. Today 20, 135–140
24. Whaley, K., and Ruddy, S. (1976) J. Exp. Med. 144, 1147–1163
25. Pangburn, M. K., Schreiber, R. D., and Muller-Eberhard, H. J. (1977) J. Exp. Med. 146, 257–270
26. Gordon, D. L., Kaufman, R. M., Blackmore, T. K., Kwong, J., and Lublin, D. M. (1995) J. Immunol. 155, 348–356
27. Kühn, S., and Zipfel, P. F. (1996) Eur. J. Immunol. 26, 2383–2387
28. Bautema, R., and Meri, S. (1999) Microbes Infect. 1, 785–784
29. Lindahl, G., Sjöbring, U., and Johansson, E. (2000) Curr. Opin. Immunol. 12, 44–51
30. Pangburn, M. K., and Müller-Eberhard, H. J. (1978) Proc. Natl. Acad. Sci.
30. Koistinen, V., Wessberg, S., and Leikola, J. (1989) *Comp. Inflamm.* 6, 270–280
31. Kuhn, S., Skerka, C., and Zipfel, P. F. (1995) *J. Immunol.* 155, 5683–5689
32. Prödinger, W. M., Hellwage, J., Spruth, M., Dierich, M. P., and Zipfel, P. F. (1998) *Biochem. J.* 331, 41–47
33. Hellwage, J., Jokiranta, T. S., Koistinen, V., Vaarala, O., Meri, S., and Zipfel, P. F. (1999) *FEBS Lett.* 462, 345–352
34. Jokiranta, T. S., Hellwage, J., Koistinen, V., Zipfel, P. F., and Meri, S. (2000) *J. Biol. Chem.* 275, 27657–62
35. Kuhn, S., and Zipfel, P. F. (1995) *Gene* (Amst.) 162, 225–229
36. Juntila, J., Peltonan, M., Soini, H., Marjamaki, M., and Viljanen, M. K. (1999) *J. Clin. Microbiol.* 37, 1361–1365
37. Schmid, B., Mullerlegger, R. R., Stockenhuber, C., Seyer, H. P., Hoeldl, S., Luger, A., and Kerl, H. (1996) *J. Clin. Microbiol.* 34, 1359–1363
38. Soini, H., Bottger, E. C., and Viljanen, M. K. (1994) *J. Clin. Microbiol.* 32, 2944–2947
39. Lam, T. T., Nguyen, T. P., Montgomery, R. R., Kantor, F. S., Fikrig, E., and Flavell, R. A. (1994) *Infect. Immun.* 62, 2079–2084
40. Wahlberg, P., Granlund, H., Nyman, D., Panelius, J., and Seppälä, I. (1993) *Ann. Med.* 25, 349–352
41. Jokiranta, T. S., Zipfel, P. F., Hakulinen, J., Kuhn, S., Pangburn, M. K., Tamerius, J. D., and Meri, S. (1996) *FEBS Lett.* 393, 297–302
42. Heimer, G. V., and Taylor, C. E. (1974) *J. Clin. Pathol.* 27, 254–256
43. Zipfel, P. F., Jokiranta, T. S., Hellwage, J., Koistinen, V., and Meri, S. (1999) *Immunopharmacology* 42, 53–60
44. Horstmann, R. D., Sievertsen, H. J., Knoblach, J., and Fischetti, V. A. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 1657–1661
45. Johnson, E., Berggard, K., Kotarsky, H., Hellwage, J., Zipfel, P. F., Sjöbring, U., and Lindahl, G. (1998) *J. Immunol.* 161, 4894–4895
46. Kotarsky, H., Hellwage, J., Johnsson, E., Skerka, C., Svensson, H. G., Lindahl, G., Sjöbring, U., and Zipfel, P. F. (1998) *J. Immunol.* 160, 3349–3354
47. Neelameg, C., Geelen, S. P., Aerts, P. C., Daha, M. R., Molines, T. E., Roord, J. J., Postuma, G., van Dijk, H., and Fleer, A. (1999) *Infect. Immun.* 67, 4517–4524
48. Ram, S., Sharma, A. K., Simpson, S. D., Gulati, S., McQuillen, D. P., Pangburn, M. K., and Rice, P. A. (1998) *J. Exp. Med.* 187, 743–752
49. Ram, S., McQuillen, D. P., Gulati, S., Elkins, C., Pangburn, M. K., and Rice, P. A. (1998) *J. Exp. Med.* 188, 671–680
50. Schwan, T. G., and Piesman, J. (2000) *J. Clin. Microbiol.* 38, 382–388
51. Stevenson, B., Schwan, T. G., and Rosa, P. A. (1995) *Infect. Immun.* 63, 4535–4539
52. Ramamoorthy, R., and Philipp, M. T. (1998) *Infect. Immun.* 66, 5119–5124
53. Nguyen, T. P., Lam, T. T., Barthold, S. W., Telford, S. R., III, Flavell, R. A., and Fikrig, E. (1994) *Infect. Immun.* 62, 2079–2084
54. Guo, B. P., Norris, S. J., Rosenberg, L. C., and Hook, M. (1995) *Infect. Immun.* 63, 3467–3472
55. Fikrig, E., Feng, W., Barthold, S. W., Telford, S. R., III, and Flavell, R. A. (2000) *J. Immunol.* 164, 5344–5351
56. Probert, W. S., and Johnson, B. J. (1998) *Mol. Microbiol.* 30, 1003–1015
57. Kraicz, P., Skerka, C., Kirschfink, M., Brade, V., and Zipfel, P. F. (2000) *Immunopharmacology* 498, 57 (abst.)