The human complement regulatory factor-H-like protein 1, which represents a truncated form of factor H, displays cell-attachment activity

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Complement factor H (FH) and factor-H-like protein 1 (FHL-1) are human plasma proteins with regulatory functions in the alternative pathway of complement activation. FH and FHL-1 are organized in repetitive elements termed short consensus repeats (SCRs) and the seven SCRs of FHL-1 are identical with the N-terminal domain of the 20 SCRs of FH. The fourth SCR of both proteins (SCR 4) includes the sequence Arg-Gly-Asp (RGD), a motif that is responsible for the major adhesive activity of matrix proteins like fibronectin. A synthetic hexapeptide with the sequence ERGDAV derived from the RGD domain of FH/FHL-1 interferes with cell attachment to a fibronectin matrix. Although the identical motif is present in both FH and FHL-1, only FHL-1 acts as a matrix for cell spreading and attachment, thus the two proteins differ in function. The adhesive activity of FHL-1 is localized to the RGD-containing SCR 4 by the use of recombinant fragments. All three analysed anchorage-dependent cell lines (CCl64, C32 and MRC-5) adhere to an FHL-1 matrix. The use of synthetic peptides in competition assays, on either FHL-1-derived or fibronectin matrices, shows that the cellular receptors binding to the FH/FHL-1-derived RGD motif are related to or identical with integrin receptors which interact with fibronectin. The identification of a functional adhesive domain in the FH/FHL-1 sequence demonstrates, at least for FHL-1, a role in cell attachment and adhesion.

INTRODUCTION

Complement factor H (FH) is a 150 kDa human plasma protein that plays an essential regulatory role in the alternative pathway of complement activation. The FH gene encodes a protein of 1234 amino acids. The 42 kDa human factor-H-like protein 1 (FHL-1) is encoded by an mRNA which is derived from the FH gene by means of alternative splicing [1,2]. The total length of the FHL-1 cDNA is 1644 bp, representing a protein composed of 449 amino acids. FHL-1 represents the N-terminal region of FH and the two proteins are identical over 445 amino acids.

The plasma forms of FH and FHL-1 are exclusively organized into repetitive elements, termed short consensus repeats (SCRs). FH and FHL-1 are composed of 20 and 7 consecutive SCRs respectively. Each SCR, which is about 60 amino acids in size, contains four conserved cysteine residues and several additional characteristic amino acids. Ultrastructural studies show that SCRs are individually folding domains that form a globular bead-like structure [3,4]. On the basis of such an organization, an elongated, flexible structure is predicted for SCR-containing proteins like FH and FHL-1. This kind of structure, where the flexibility of the protein results in a loop with the two ends of the protein folded back on themselves, was confirmed by electron microscopy [5,6].

FHL-1 is identical in sequence with SCRs 1–7 of FH, but with four additional amino acids added at the C-terminal end. Both FHL-1 and FH possess cofactor activity in factor-I-mediated cleavage of C3b [1,7] and decay-accelerating activity, as they support the dissociation of the C3b·Bb complex [8]. It has been demonstrated that the first four N-terminal SCRs (SCRs 1–4) of FH and FHL-1 are essential and sufficient for both these activities [8–10]. In addition, a heparin-binding site has recently been localized to SCR 7 of FH and FHL-1 [11].

The amino-acid sequence motif Arg-Gly-Asp (RGD) is present in a number of adhesive matrix proteins such as fibronectin, fibrinogen, vitronectin and von Willebrand factor [12–14] and has been shown to be responsible for their cell-adhesive properties. Short synthetic RGD-containing peptides can mimic these properties, further suggesting that this tripeptide domain is important for mediating cell adhesion [15,16]. The interaction of the RGD-containing ligand with its corresponding receptor is specific, as a peptide with the modified sequence RGE has no activity. All known RGD-binding receptors are members of the integrin family of cell-adhesion molecules [13,17]. The mechanism and specificity of integrin binding to the RGD-containing ligands remains to be established, as regions outside of the RGD-containing domain also seem to be involved in receptor interaction [18]. The presence of an RGD motif within a linear amino-acid sequence does not necessarily imply that the protein can interact with cellular integrin receptors or that it plays a role in cell adhesion. For example, it has been reported that the RGD domain of the complement protein C3 is not necessary for binding to the cellular integrin receptor CR3 [19,20].

Sequence analysis identifies the RGD motif within SCR 4 of both FH and FHL-1. The presence of this motif suggests that these two complement proteins can modulate cell adhesion [13,21]. Given the structure determined for single SCRs, the RGD domain is located within a loop at a position where it is exposed to solvent, suggesting that this domain is accessible to other proteins or cellular receptors [3,4,6]. The RGD motif of FH is conserved in evolution. It is found at a corresponding position in human and mouse FH [22], and also in a protein of the bony fish barred sandbass (*Paralabrax nebulifer*), which is similar in both structure and amino-acid sequence to human FH [23]. This evolutionary conservation is indicative of a conserved function.

Abbreviations used: FH, factor H; FHL-1, factor-H-like protein 1; SCR, short consensus repeat.

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Given the existence of the RGD motif in SCR 4 of FH and FHL-1, we considered whether these proteins play a role in cell adhesion. Here we demonstrate that a synthetic peptide with the RGD motif of FH/FHL-1 interferes with cell attachment to a fibronectin matrix and that FHL-1, but not FH, serves as an attachment matrix for anchorage-dependent cells.

EXPERIMENTAL

Cloning of recombinant fragments of FHL-1

Recombinant proteins representing selected domains of human FHL-1 and FH were synthesized using the baculovirus expression system. Construction and expression of the various deletion mutants has been described recently [8,10]. Briefly, recombinant FHL-1 protein similar to SCR 1–7 of FH was derived from a full-length FHL-1 cDNA (kindly provided by Dr. C. Skerka, Bernhard Nocht Institute, Hamburg, Germany). Fragments representing SCR 1–3, SCR 1–4, SCR 2–4 or SCR 3–5 were amplified by PCR, subcloned into baculovirus expression vector pBSV-8His, and sequences were confirmed by DNA sequencing [24]. Recombinant virus, obtained by homologous recombination, was used to infect insect cells. All recombinant proteins included a C-terminal His-tag to allow direct purification from the culture medium by Ni^{2+}-chelate chromatography [24,25].

Expression and purification of recombinant fragments of FHL-1

*Sf*9 cells (3×10^6) were grown in a 140 mm cell-culture dish in 22 ml of Express medium (Boehringer Ingelheim BioWhittaker, Verviers, Belgium) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (250 ng/ml) and infected with recombinant virus using a multiplicity of infection of 5. The culture supernatant was harvested 10 days after infection and the recombinant proteins were purified by immobilized metal-affinity chromatography as described [24]. Briefly, the Ni^{2+}-chelate resin (Qiagen) was precipitated by centrifugation and washed twice with 10 vol. of buffer A [0.5 M NaCl/20 mM Tris/HCl (pH 7.9)/5 mM imidazole], followed by 1 vol. of buffer B [0.5 M NaCl/20 mM Tris/HCl (pH 7.9)/60 mM imidazole]. The recombinant protein was eluted with buffer C [0.5 M NaCl/20 mM Tris/HCl (pH 7.9)/1 M imidazole]. The resin was restored by washing with 10 vol. of buffer D containing 100 mM EDTA. Purified recombinant proteins were dialysed against 50 mM Tris/HCl, pH 7.5 overnight and the protein concentration was measured by the method of Bradford [26].

SDS/PAGE analysis

The purified proteins were analysed by SDS/PAGE [27] using 12 and 15 % separating gels. Proteins were revealed by silver staining [28] or Western blotting (not shown) [29]. Pre-stained molecular-weight markers were purchased from Bio-Rad.

Cell lines and culture

Insect cells (*Sf*9 cells from *Spodoptera frugiperda*) were used for infection with recombinant baculoviruses. Cells were grown at 27 °C at 95 % humidity in Grace's Medium (Boehringer Ingelheim BioWhittaker) containing 10 % fetal-calf serum (Gibco–BRL). The following mammalian cell lines were used: mink epithelial-like cell-line CCL64 was kindly provided by Professor B. Fleischer (Bernhard Nocht Institute, Hamburg, Germany), human melanoma C32 cells were kindly provided by M. Freyer (Bernhard Nocht Institute, Hamburg, Germany) and human fibroblast-like MRC-5 were purchased from A.T.C.C. Cells were grown in RPMI 1640 (Boehringer Ingelheim BioWhittaker), supplemented with 10 % fetal-calf serum (Gibco–BRL), penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (250 ng/ml) in an atmosphere of 5 % CO_2 at 95 % humidity.

Cell spreading and adhesion assays

Cells were grown to near-confluency and harvested by brief exposure to trypsin/EDTA (Gibco–BRL). Upon recovery in serum-free medium, the cells were washed and resuspended in plain RPMI 1640 medium. Chamber slides (Nunc) were coated overnight at 4 °C with purified serum proteins FH (Quidel), fibronectin (Biomol, Hamburg, Germany) or BSA (Sigma). In addition, recombinant FHL-1 and recombinant proteins representing SCR 1–3, SCR 1–4, SCR 2–4 and SCR 3–5 were used. In all cases, the protein concentration was 5 μg/cm^2. The slides were blocked using BSA dissolved in PBS (1 mg/ml) at 37 °C for 1 h, and then were washed twice with PBS. Cells (1×10^6) resuspended in 1 ml of plain RPMI 1640 medium were added to the chambers. In inhibition experiments, the synthetic peptides G<u>RGD</u>SP and G<u>RGE</u>SP (Biomol) or E<u>RGD</u>AV (synthesized by Dr. Kullmann, Institut für Zellbiochemie, Hamburg, Germany) were added to the cell suspension at a final concentration of 50 μM. The effect of chelating agents was analysed by addition of 2.5 mM EGTA or EDTA. After 1 h incubation at 37 °C, cells were fixed in PBS containing 2 % (v/v) glutaraldehyde, 5 % (v/v) formaldehyde and 5 % (w/v) sucrose [30]. The number of spread cells was counted under the microscope. For photographic documentation, cells were stained with Giemsa solution (Merck). All experiments were repeated at least five times, and a minimum of 500 cells were counted in each.

RESULTS

Inhibition of cell spreading on a fibronectin matrix by synthetic peptides

The extracellular glycoprotein fibronectin promotes cell attachment and spreading, as demonstrated by the adherence of epithelial CCl64 cells to a fibronectin matrix (Figures 1 and 2). As shown previously, the importance of the RGD motif for cell attachment and spreading is demonstrated by inhibition experiments with RGD-containing peptides [15,16]. A hexapeptide with the RGD motif and flanking fibronectin-derived amino acids (G<u>RGD</u>SP) inhibited cell attachment in a dose-dependent manner. The specificity of the RGD motif for attachment is demonstrated by the peptide G<u>RGE</u>SP (RGE). This peptide,

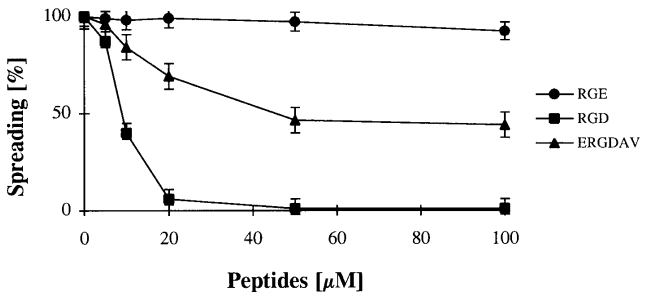

Figure 1 Inhibition of cell attachment by synthetic peptides

Dose-dependent inhibition of attachment of CCl64 cells to a fibronectin matrix was analysed in the presence of the indicated concentrations of the fibronectin-derived hexapeptide G<u>RGD</u>SP (RGD), the FH/FHL-1-derived peptide E<u>RGD</u>AV (ERGDAV) or the modified fibronectin-derived hexapeptide with the sequence G<u>RGE</u>SP (RGE). Results are means and the bars represent $\pm$S.D. ($n = 5$)

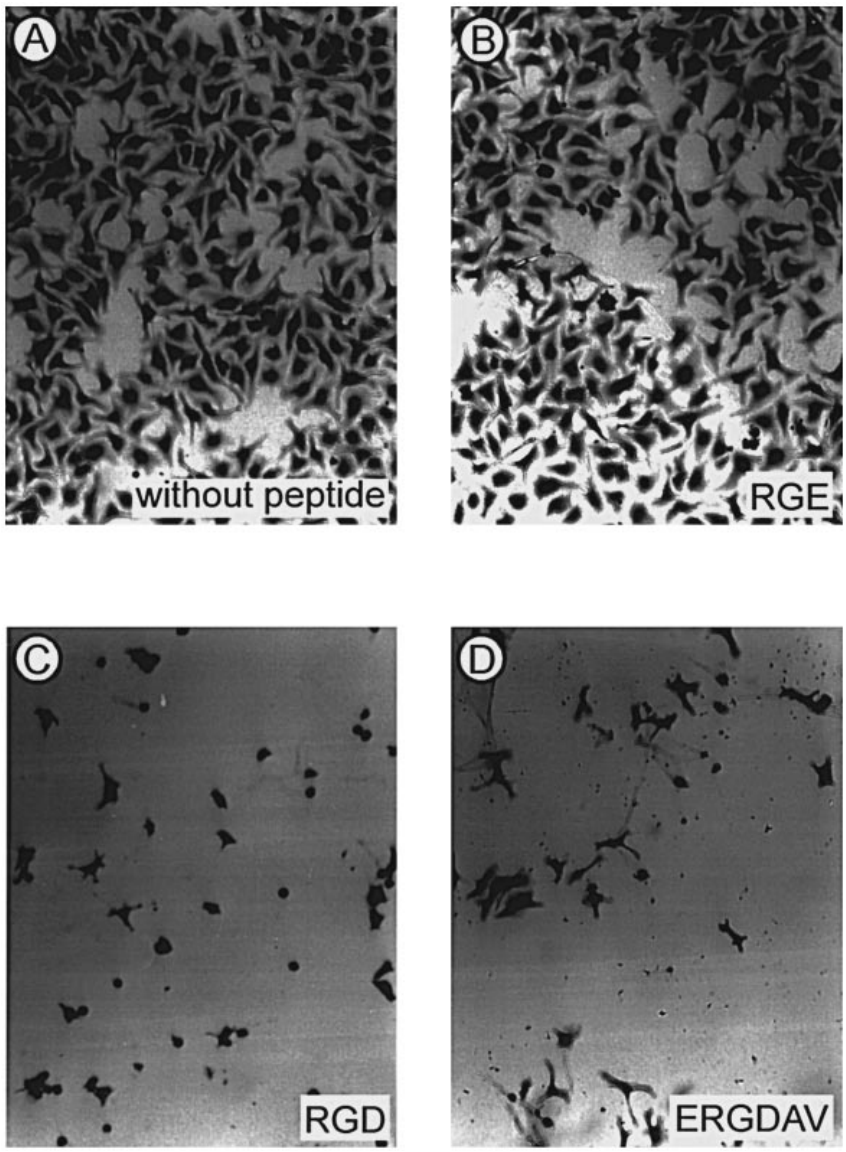

Figure 2 Effect of synthetic peptides on the morphology of cells attached to a fibronectin matrix

CCl64 cells were allowed to attach to a surface coated with fibronectin. (**A**) In the absence of synthetic peptides, attached cells show a fibroblast-like morphology; (**B**) the modified fibronectin-derived peptide GRGESP (RGE) does not effect cell attachment; (**C**) the fibronectin-derived GRGDSP (RGD) peptide does interfere with cell attachment, demonstrated by the reduced number and the different morphology of attached cells; (**D**) the FH/FHL-1-derived peptide (ERGDAV) reduced the number and altered the morphology of attached cells.

which includes an Asp-to-Glu replacement, did not interfere with cell spreading to a fibronectin matrix (Figures 1 and 2).

In order to prove whether FH and FHL-1 may also play a role in cell adhesion, a synthetic hexapeptide, derived from the FH/FHL-1 amino acid sequence (ERGDAV), was analysed for its effect in cell spreading. Similarly to GRGDSP, ERGDAV did inhibit attachment of CCl64 cells to a fibronectin matrix. However, this inhibitory effect was less pronounced; ERGDAV showed 55% inhibition at 50 μM, compared with 95% obtained with the GRGDSP peptide (RGD).

Epithelial cells attached to a fibronectin matrix showed a morphology typical for anchorage-dependent cells (Figure 2A). GRGESP did not affect either the morphology or the number of attached cells (Figure 2B). However, both GRGDSP and ERGDAV peptides affected cell attachment; the inhibitory activity of these was demonstrated by the reduced number of adherent cells and by morphological changes (Figures 2C and 2D).

Effect of FH and FHL-1 on cell spreading and attachment

As an ERGDAV hexapeptide of FH/FHL-1 was able to interfere with cell attachment to a fibronectin matrix, we tested whether FH or FHL-1 themselves were also able to promote cell spreading and attachment. The structures of FH and FHL-1 are shown in Figure 3(A). FH is organized into 20 SCRs, and FHL-1, which represents the N-terminal seven SCRs of FH, has four amino acids added at the C-terminal end. In both proteins, the RGD motif is located within SCR 4.

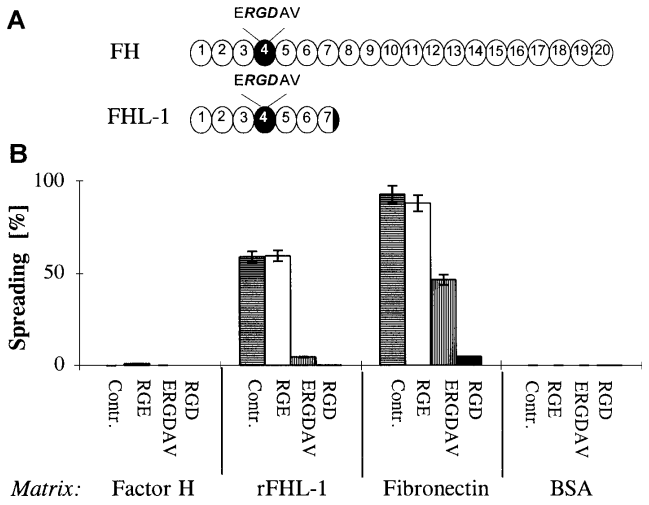

Figure 3 Cell spreading to an FH or FHL-1 matrix

(**A**): Schematic structure of the human complement regulatory proteins FH and FHL-1. SCRs are shown in their globular structure and are numbered consecutively starting at the N-terminus. The RGD-containing SCR 4 of FH and FHL-1 are highlighted and the four different amino acids at the C-terminal end of FHL-1 are indicated. (**B**): Effect of FH and FHL-1 on cell spreading and attachment. Chamber slides were coated with the indicated proteins and cell attachment of CCl64 cells was analysed. Specificity of the interaction was shown by competition with synthetic hexapeptides with the modified fibronectin-derived (RGE), the FH/FHL-1-derived (ERGDAV) or with the fibronectin-derived domain (RGD). Fibronectin and BSA were used as positive and negative controls respectively. Results are means, and the bars represent $\pm$S.D. ($n = 5$).

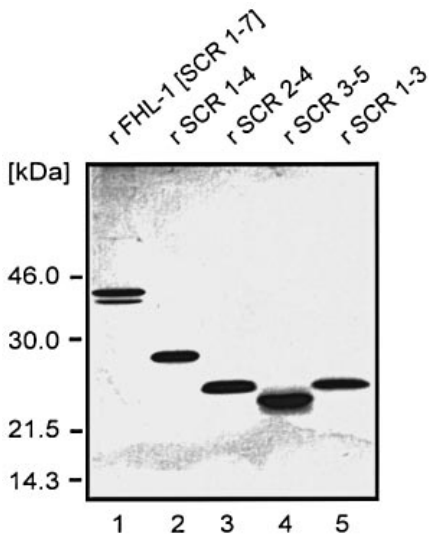

Figure 4 Recombinant fragments used for cell attachment analysis

Various fragments representing the N-terminal region of FH/FHL-1 were expressed in their native configuration preserving the structure and organization of the SCR domains. Recombinant proteins were expressed in the baculovirus expression system as secreted His-tagged proteins. Purified recombinant proteins were separated by SDS/PAGE and analysed by silver staining.

FH and FHL-1 were used as a matrix to study their role in cell attachment and spreading. CCl64 cells did not attach to a FH matrix; however, an FHL-1 matrix allowed cell spreading and attachment (Figure 3B). Compared with the fibronectin matrix, the FHL-1 matrix showed a reduced spreading capacity of about 59%. CCl64 cells did not attach to BSA (used as a negative control). The specificity of the interaction between the cells and their protein matrix was shown by inhibition with the various hexapeptides. GRGESP did not effect cell attachment on either an FHL-1 or a fibronectin matrix. As observed previously, the two RGD-containing hexapeptides showed different activities on a fibronectin matrix. However, on the FHL-1 matrix both GRGDSP and ERGDAV had similar inhibitory activities.

Localization of the adhesion domain of FHL-1

The formation of intramolecular disulphide bonds is particularly important for the three-dimensional structure of the individually folding SCR domain. In order to obtain properly folded recombinant domains of FH/FHL-1, we employed the baculovirus system. It has recently been demonstrated that this eukaryotic expression system is particularly useful for expression of properly processed, functionally active SCR domains [24].

In order to localize the adhesion domain of FHL-1, recombinant FHL-1 and various RGD-containing fragments (rSCR 1–4, rSCR 2–4, rSCR 3–5) or a fragment lacking SCR 4 (rSCR 1–3) were expressed. Recombinant proteins were directly purified to homogeneity from the culture medium using immobilized metal-affinity chromatography and separated by SDS/PAGE to assess their purity by silver staining (Figure 4).

The capacity of the individual recombinant fragments to serve as matrix for cell attachment was analysed. rFHL-1 in its entirety and all recombinant proteins which included SCR 4 (rSCR 1–4, rSCR 2–4, rSCR 3–5) displayed spreading activity for CCl64 cells (Figure 5). Similarly with the negative control BSA, the recombinant protein lacking SCR 4 (rSCR 1–3) had no effect on cell spreading and attachment. These results revealed the importance of SCR 4 for cell attachment. The inability of rSCR 1–3 to serve as a matrix for cell attachment demonstrated that the His-tag that was added to the recombinant proteins did not interfere with cell spreading. The specificity of interaction between the cellular receptors and the various FHL-1-derived matrices was confirmed by inhibition experiments with GRGDSP and ERGDAV peptides and by the inability of GRGESP to interfere with the attachment. On an FHL-1 or FHL-1-derived matrix, the inhibitory activities of both RGD-containing peptides were similar. Compared with the complete FHL-1 protein, all three FHL-1-derived proteins which included SCR 4 were more effective in cell attachment. Some 95% of cells adhered to the rSCR 3–5 matrix (Figure 5), a protein which has the RGD-containing SCR flanked on each side by an SCR. The activity of this particular protein was equivalent to that of fibronectin. Taken together, the use of recombinant fragments of FHL-1, which have a native organization and the SCR structure conserved, demonstrated that the RGD-containing SCR 4 is important for cell attachment.

Spreading of different cell lines on an FHL-1-derived adhesion matrix

Adherent cells can express different types of adhesion receptors. We therefore compared the spreading activity of three mammalian cell lines with an FHL-1-derived matrix (Table 1). The mink lung cell-line CCl64, which displays fibroblast-like morphology, showed the highest spreading rate (94%), whereas the human melanoma cell-line C32 showed the lowest (44%). Adherence of human lung fibroblasts MRC-5 to the FHL-1 matrix was almost as efficient as that of CCl64 cells (86%). The specificity of cell spreading was confirmed by the inability of

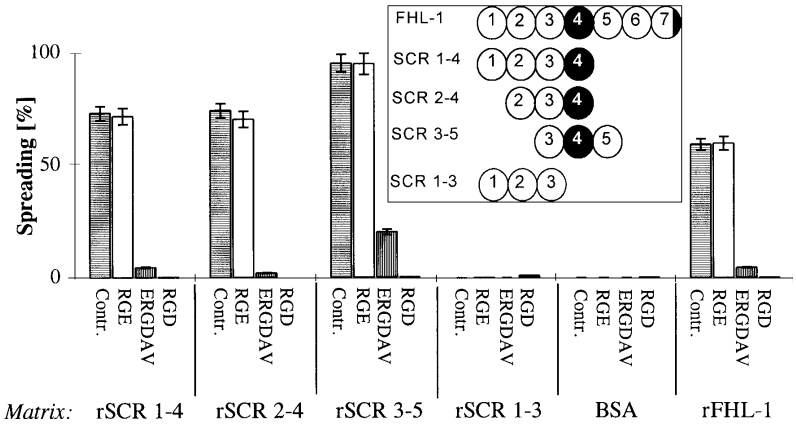

Figure 5 Cell attachment of CCl64 cells to recombinant FH/FHL-1 fragments

The inserted box shows the SCR structure of the various recombinant fragments that were used for cell attachment analysis either including (rFHL-1, rSCR 1–4, rSCR 2–4, rSCR 3–5) or excluding (rSCR 1–3) the RGD-containing SCR 4. Chamber slides were coated with the indicated recombinant fragments or with BSA and attachment of CCl64 cells was analysed. The specificity of the cellular interaction was analysed by competition with different synthetic peptides. For explanation of the synthetic peptides compare legend with Figure 1. Results are means ± S.D. ($n = 5$)

Table 1 Attachment of anchorage-dependent cells to an FH/FHL-1-derived matrix

Glass surfaces were coated with an FHL-1-derived matrix (rSCR 3–5) at a concentration of 5 μg/cm^2. Attachment of various cell lines were compared using the mink cell line CCl64, the human melanoma cell line C32 and human fibroblasts MRC-5. Results are means ± S.D. ($n = 5$).

Cell line	Spreading rate ± S.D. (%)
CCl64	94.0 ± 1.3
C32	44.4 ± 15.1
MRC-5	86.0 ± 6.2

Table 2 Inhibition of cell attachment of human MRC-5 fibroblasts

Inhibition of cell attachment of human MRC-5 fibroblasts to an FH/FHL-1-derived matrix (rSCR 3–5). Various synthetic peptides as explained in Figure 1 were used for inhibition.

Peptide	Spreading rate ± S.D. (%)
Control	74.8 ± 6.2
RGE	70.1 ± 6.4
ERGDAV	2.0 ± 1.2
RGD	0.1 ± 1.0

GRGESP to interfere with cell attachment. GRGDSP and ERGDAV peptides displayed similar inhibitory effects on cell spreading (Table 2).

Effect of bivalent cations on cell spreading

To further confirm that the effect of FHL-1 is mediated by binding of the RGD domain to an integrin receptor, we asked whether chelating agents can interfere with spreading activity. The importance of bivalent cations for cell spreading was therefore analysed in the presence and absence of EDTA and EGTA. On a matrix of FHL-1 consisting of SCR 3–5, cell spreading was typically observed for 94.8 % of the cells, whereas EDTA interfered substantially with cell attachment, reducing the fraction of attached cells to 10.0 % (Table 3). The effect of EGTA was even more pronounced, the fraction of spread cells reduced to < 1 %.

Table 3 Inhibition of attachment by chelating agents

Chelating agents inhibit attachment of CCl64 cells to a matrix of SCR 3–5. Cell attachment to a matrix consisting of rSCR 3–5 in the absence (Control) or presence of either EGTA (2.5 mM) or EDTA (2.5 mM). Results are means ± S.D. ($n = 5$).

	Spreading rate ± S.D. [%]
Control	94.8 ± 4.7
EGTA	1.0 ± 0.5
EDTA	10.0 ± 0.9

DISCUSSION

The complement regulatory protein FH and FHL-1, a product translated from an alternatively spliced transcript of the FH gene [1], display the sequence motif RGD in their fourth SCR. This tripeptide plays an essential role in cell adhesion [15]. Here we demonstrate that the synthetic peptide ERGDAV, which represents six amino acids of the FH/FHL-1 sequence, interferes with cell adhesion to a fibronectin matrix. Although the RGD motif is present in both FHL-1 and FH at identical positions, our experiments showed that only FHL-1 promotes cell attachment.

Our results identify a novel function in cell spreading and attachment for FHL-1. This adhesive function is confirmed by the ability of an FH/FHL-1-derived peptide (ERGDAV) to interfere with cell spreading and binding of anchorage-dependent cells to a fibronectin matrix. Compared with the fibronectin-derived peptide GRGDSP, which was used as a positive control, ERGDAV shows a lower activity. However, the specificity of the attachment is shown by the GRGESP peptide, which does not interfere with cell spreading. A matrix consisting of an intact recombinant FHL-1 protein also promotes cell adhesion. Com-

pared with fibronectin, the FHL-1 matrix has a reduced activity of about 50 %. The involvement of the RGD-containing SCR 4 in cell attachment is demonstrated by the use of recombinant fragments which were expressed in their native SCR-domain structure. Recombinant proteins which include the RGD-containing SCR 4 (rSCR 1–4, rSCR 2–4, rSCR 3–5) promote attachment in cell-spreading assays, whereas a protein which lacks SCR 4 (rSCR 1–3) has no such activity. The number of cells adhering to the FHL-1-derived SCR 3–5 matrix is comparable with that of fibronectin, demonstrating that SCR 4 is an efficient mediator of cell attachment.

The activity of SCR 4 in cell spreading increases with decreasing size of the protein and number of SCRs. SCR 3–5, composed of three SCRs, displays a spreading activity that is comparable with that of fibronectin (95 %). FHL-1 is composed of seven SCRs and only 59 % of the cells adhere to this matrix. FH, containing 20 SCRs, has no activity in the adhesion assay (Figure 3). Although SCR 4 promotes cell spreading, the different activities of the various SCR-containing proteins show that the accessibility of the RGD domain is influenced by the presence of other SCRs. A structural analysis of FH has demonstrated that SCRs can interact with each other and that the head and tail domains can fold back on themselves forming a loop [5,6]. In such a configuration, the RGD domain seems to be masked and inaccessible to interacting proteins. Whether FH has adhesive functions is currently unclear. The RGD-containing domain within SCR 4 is functional, as demonstrated for FHL-1 and the truncated proteins. Thus for FH it is possible that interaction of the head and tail domains results in an inaccessible RGD domain. A conformational transition of the protein is required to expose the RGD domain and make it accessible to cellular receptors.

FHL-1 is a member of a family of structurally and immunologically related plasma proteins. In addition to FHL-1 and FH, four factor-H-related proteins (FHR-1 to FHR-4) have been identified [31,32]. FHL-1 is a plasma protein with multiple functions. Similarly to FH, it plays a regulatory role in the alternative complement pathway. The protein acts as cofactor for factor-I-mediated degradation of C3b and promotes decay acceleration of the C3b·Bb complex [8,10]. As demonstrated in here, FHL-1 promotes cell spreading of anchorage-dependent cells. FHL-1 is not the only protein which combines complement regulatory activity and cell-attachment functions: similarly, the adhesive human plasma glycoprotein vitronectin (or serum-spreading factor, S-protein) acts as an inhibitor of complement-mediated cytolysis [14]. Vitronectin and FH are present in plasma at a similar concentration of 500 μg/ml. The concentration of the FHL-1 protein is lower, at about 10–50 μg/ml ([8]; J. Hellwage, S. Kühn and P. F. Zipfel, unpublished work) but still within a physiological range comparable with that of factor I (35 μg/ml) or clusterin (50 μg/ml).

The cell-attachment activity of FHL-1 resides in the RGD epitope, which is shared by other glycoproteins, such as vitronectin, fibronectin, fibrinogen, thrombospondin, von Willebrand factor and others [15]. The RGD domain of each of these adhesive proteins is recognized by at least one member of a family of structurally related receptors termed integrins. Integrins are heterodimeric membrane glycoproteins with two membrane-spanning subunits that are expressed on a variety of cell types [13]. As adhesion proteins, they function as major receptors for the extracellular matrix and as cell adhesion molecules. Biological processes in which integrins play an important role include platelet aggregation, inflammation, immune functions, wound healing and tissue migration. They are also involved in cellular signalling [33,34]. The N-terminal region of the integrin α-subunit contains three or four bivalent-cation-binding sites, and complexed cations are essential for ligand binding [13]. The fact that all analysed cell lines interact with the rFHL-1-derived matrix through integrin receptors is also confirmed by the requirement for bivalent cations. Chelating agents like EDTA and EGTA almost completely reduced cell attachment to the FHL-1 matrix (Table 3). Thus, in addition to the essential role of the RGD motif, the requirement for bivalent cations is a strong indication of the involvement of integrin receptor binding to FHL-1.

Previous studies have reported that FH itself binds to specific cellular receptors, which have been identified in a variety of cell types, such as B cells, polymorphonuclear monocytes and others [35–37]. Similarly, FH induced oxidative metabolism and the release of IL-1β in monocytes [38,39]. Approx. 6×10^4 binding sites for FH have been calculated on human polymorphonuclear leucocytes and binding is also dependent on bivalent cations [40]. No cellular receptor for FH has yet been isolated. The reason why purified FH does not always interact with its cellular receptors is unclear. In addition to conformational changes, two distinct forms of FH (Φ_1 and Φ_2) have been described [41] which differ in hydrophobicity and in their ability to bind to cellular receptors.

The receptor-binding site of FH has been localized to the 38 kDa tryptic fragment, which represents the N-terminal SCRs 1–6 [40]. This result obtained for FH is in agreement with the observation reported here for recombinant fragments of FH/FHL-1. In this paper the RGD domain of SCR 4 was demonstrated to be important for receptor interaction, whereas previously it was suggested that SCR 5 is involved in receptor binding [40]. The importance of SCR 5 was confirmed by inhibition experiments, as monoclonal antibodies directed against SCR 5 blocked receptor binding. However, these results do not contradict our hypothesis that the RGD domain within SCR 4 is the major site interacting with a putative FH receptor. Monoclonal antibodies of the IgG1 subtype are large proteins with a size comparable with that of FH. Thus precise mapping of functional domains within a given protein is problematic, as an antibody which binds to SCR 5 may render the RGD domain in the adjacent SCR 4 inaccessible to cellular receptors, or alternatively the antibody may produce steric effects at sites distal to the binding epitope.

It can be assumed that interaction of the RGD domain with the cellular receptor inhibits the complement regulatory activities of FH and FHL-1. We have recently demonstrated that SCRs 1–4 of FH/FHL-1 are essential for both decay acceleration and cofactor activity [8,10]. The role of SCR 4 in complement regulation is highlighted by a mutant that lacks this particular domain. The truncated protein, consisting of SCR 1–3, lacks functional activity completely. Thus upon binding to the cellular receptor via the RGD domain of SCR 4, FH and FHL-1 seem to lose complement-regulatory activities. However, in their receptor-bound state, both proteins play a role in inflammatory reactions by enhancing attachment of phagocytotic cells. By binding to C3b deposited on target cells and integrin receptors on effector cells simultaneously, FH and FHL-1 may enhance binding of polymorphonuclear cells to their target surfaces. Furthermore, two additional cell-binding domains have been identified for FH. These domains, which interact with glycosaminoglycans and heparin, have been localized near to or in SCRs 7 and 13 [11,42,43]. The identification of the cellular FH and FHL-1 receptor will be necessary to characterize in detail the additional biological role(s) of these two human plasma proteins.

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REFERENCES

1 Misasi, R., Huemer, P., Schwaeble, W., Sölder, E., Larcher, C. and Dierich, M. P. (1989) Eur. J. Immunol. **19**, 1765–1768
2 Sim, R. B., Kölble, K., McAleer, M. A., Dominguez, O. and Dee, V. M. (1993) Int. Rev. Immunol. **10**, 65–86
3 Norman, D. G., Barlow, P. N., Baron, M., Day, A. J., Sim, R. B. and Campbell, I. D. (1991) J. Mol. Biol. **219**, 717–725
4 Barlow, P. N., Norman, D. G., Steinkasserer, A., Horne, T. J., Pearce, J., Driscoll, P. C., Sim, R. B. and Campbell, I. D. (1992) Biochemistry **31**, 3626–3634
5 DiScipio, R. G. (1992) J. Immunol. **149**, 2592–2599
6 Barlow, P. N., Steinkasserer, A., Norman, D. G., Kieffer, B., Wiles, A. P., Sim, R. B. and Campbell, I. D. (1993) J. Mol. Biol. **232**, 268–284
7 Alsenz, J. D., Lambris, J., Schulz, T. F. and Dierich, M. P. (1984) Biochem. J. **224**, 389–398
8 Kühn, S. and Zipfel, P. F. (1996) Eur. J. Immunol. **26**, 2383–2387
9 Gordon, D. L., Kaufman, R. M., Blackmore, T. K., Kwong, J. and Lublin, D. M. (1995) J. Immunol. **155**, 348–356
10 Kühn, S., Skerka, C. and Zipfel, P. F. (1995) J. Immunol. **155**, 5663–5670
11 Blackmore, T. K., Sadlon, T. A., Ward, H. M., Lublin, D. M. and Gordon, D. L. (1996) J. Immunol. **157**, 348–356
12 Hynes, R. O. (1987) Cell **48**, 549–554
13 Hynes, R. O. (1992) Cell **69**, 11–25
14 Preissner, K. T. and Jenne, D. (1991) Thromb. Haemostasis **66**, 123–132
15 Pierschbacher, M. D. and Ruoslahti, E. (1984) Nature (London) **309**, 30–34
16 Hayman, E. G., Pierschbacher, M. D. and Ruoslahti, E. (1985) J. Cell Biol. **100**, 1948–1954
17 Pytela, R., Pierschbacher, M. D., Argraves, S., Suzuki, S. and Ruoslahti, E. (1987) Methods Enzymol. **144**, 475–489
18 Aota, S. I., Nagai, T. and Yamada, K. M. (1991) J. Biol. Chem. **24**, 15938–15943
19 Wright, S. D., Weitz, J. I., Huang, A. J., Levin, S. M., Silverstein, S. C. and Loike, J. D. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7734–7738
20 Taniguchi-Sidle, A. and Isenman, D. E. (1992) J. Biol. Chem. **267**, 635–643
21 Pierschbacher, M. D. and Ruoslahti, E. (1987) J. Biol. Chem. **262**, 17294–17298
22 Kristensen, T. and Tack, B. F. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 3963–3967
23 Dahmen, A., Kaidoh, T., Zipfel, P. F. and Gigli, I. (1994) Biochem. J. **301**, 391–397
24 Kühn, S. and Zipfel, P. F. (1995) Gene **162**, 225–229
25 Hochuli, E., Döbelin, H. and Schacher, E. (1987) J. Chromatogr. **422**, 177–184
26 Bradford, M. (1976) Anal. Biochem. **72**, 248–253
27 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28 Heukeshoven, J. and Dernick, R. (1986) Electrophoresis **6**, 103–112
29 Kyhse-Andersen, J. (1984) J. Biochem. Biophys. Methods **10**, 203–209
30 Zhao, Y. and Sane, D. (1993) Biochem. Biophys. Res. Commun. **192**, 575–582
31 Zipfel, P. F. and Skerka, C. (1994) Immunol. Today **15**, 121–126
32 Skerka, C., Hellwage, J., Weber, W., Tilkorn, A., Buck, F., Marti, T., Kampen, E., Beisiegel, U. and Zipfel, P. F. (1997) J. Biol. Chem. **272**, 5627–5634
33 Loftus, J. C., Smith, J. W. and Ginsberg, M. H. (1994) J. Biol. Chem. **269**, 25235–25238
34 Sjaastad, M. D., Angres, B., Lewis, R. S. and Nelson, W. J. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 8214–8218
35 Lambris, J. D. and Ross, G. D. (1982) J. Exp. Med. **155**, 1400–1411
36 Becherer, J. D., Alsenz, J., Servis, C., Myones, B. L. and Lambris, J. D. (1989) Complement Inflammation **6**, 142–165
37 Ohtsuka, H., Imamura, T., Matsushita, M., Tanase, S., Okada, H., Ogawa, M. and Kambara, T. (1993) Immunology **80**, 140–145
38 Schopf, R. E., Hamman, K. P., Scheiner, O., Lemmel, E. M. and Dierich, M. P. (1982) Immunobiology **46**, 307–312
39 Iferroudjene, D., Schouft, M. T., Lemercier, C., Gilbert, D. and Fontaine, M. (1991) Eur. J. Immunol. **21**, 967–972
40 Avery, V. M. and Gordon, D. L. (1993) J. Immunol. **10**, 5545–5553
41 Ripoche, J., Erdei, A., Gilbert, D., Salihi, A., Sim, R. B. and Fontaine, M. (1988) Biochem. J. **253**, 475–480
42 Pangburn, M. K., Atkinson, M. A. L. and Meri, S. (1991) J. Biol. Chem. **266**, 16847–16853
43 Meri, S. and Pangburn, M. K. (1994) Biochem. Biophys. Res. Commun. **198**, 52–59

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