Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7123–7127, September 1989
Immunology

Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid

(reactive lysis/reproduction/clusterin/Sertoli cell/S-protein)

DIETER E. JENNE AND J. TSCHOPP

Institut de Biochimie, Université de Lausanne, Chemin des Boveresses, CH-1066 Epalinges s/Lausanne, Switzerland

Communicated by Hans J. Müller-Eberhard, June 23, 1989 (received for review December 23, 1988)

ABSTRACT **A component of soluble terminal complement complexes was identified and affinity-purified to homogeneity by using a monoclonal antibody previously developed against the soluble C5b–9 complex. The protein, which we have designated complement cytolysis inhibitor (CLI), has a molecular mass of 70 kDa and consists of two nonidentical, disulfide-linked subunits of 35 kDa. Partial amino acid sequences determined for the amino-termini of the two subunits were identical with those of a recently characterized serum protein called SP-40,40. An almost full-length cDNA clone of 1651 base pairs was isolated from a human liver cDNA library by using long synthetic oligonucleotides as probes. The encoded amino acid sequence of CLI consists of 427 amino acid residues preceded by a 21-residue-long typical signal peptide and shows an overall 75.6% amino acid sequence homology to sulfated glycoprotein 2 (SGP-2), a major Sertoli cell-derived protein of rat testis fluid. As in SGP-2, proteolytic processing between residues 206 and 207 yields the two disulfide-linked subunits of plasma CLI. CLI and SGP-2 were shown to be orthologous single-copy genes in humans and rats by Southern blotting experiments. In addition, CLI was immunologically identified in human seminal plasma. Functional studies with purified terminal complement components showed that CLI suppresses the cytolytic potential of nascent C5b–7 complexes at physiological blood plasma concentrations (≈50 μg/ml). Its presence on the surface of mature sperm cells and its relative abundance in seminal plasma (≈250 μg/ml) suggest that CLI protects sperm cells and epithelial tissues against complement attack in the male reproductive tract.**

The complement system consists of more than 20 plasma proteins that form a cascade-like defense barrier against bacteria, viruses, virus-infected cells, parasites, and tumor cells. The terminal step in the complement cascade is initiated by cleavage of complement component C5 into C5a and C5b. The latter molecule and the complement components C6, C7, C8, and C9 assemble in that order to form a macromolecular protein complex that causes the lysis of the target cell membrane. The nascent terminal complement complexes bind to the target membrane and insert into the lipid bilayer at the state of C5b–7 (1–4).

The mechanisms that control the terminal complement pathway on host cells have been investigated mainly at the level of regulatory membrane factors of the target cell (5–7). However, the question of why the cytolytic potential of the nascent terminal complement complex rapidly decays in plasma has not been answered. At present, S-protein/vitronectin is regarded as the major inhibitory plasma protein that restricts the C5b–9 attack to the complement-activating target cell membrane (2, 3, 8, 9). Several other plasma components—high density lipoproteins, the apolipoproteins A1 and A2 (10), complement component C8 (11), antithrombin III (12), and a poorly characterized murine plasma factor (13)—have also been inferred as inhibitors of complement-mediated cytolysis. However, little is known about the relative importance of these different plasma proteins in protecting bystander cells from C5b–9 attack under *in vivo* conditions.

During the course of preparing monoclonal antibodies against S-protein that had been isolated from soluble terminal complement (SC5b–9) complexes (14), a monoclonal antibody (II-F4) was obtained that reacted with another, unknown protein of the SC5b–9 complex. This protein has a molecular size of 70 kDa, similar to intact S-protein under nonreducing conditions, but is otherwise not related to S-protein. It copurifies with S-protein when the latter is dissociated from fluid-phase complement complexes by deoxycholate treatment (14, 15). In view of the structural and functional properties described below we have named this protein cytolysis inhibitor (CLI). Here, we report that CLI is a potent inhibitor of the terminal complement pathway and is also found at high concentrations in human seminal plasma.*

EXPERIMENTAL PROCEDURES

Protein Purification. A murine monoclonal antibody developed previously (14, 15) was used to affinity-purify CLI from human EDTA-treated plasma. Three milligrams of IgG was purified from ascites fluid on a protein A-Sepharose column and coupled to 1.5 ml of CNBr-activated Sepharose according to the manufacturer's instructions (Pharmacia). Twenty milliters of human EDTA-treated plasma was diluted with 10 ml of Tris-buffered saline (10 mM Tris·HCl, pH 7.4/150 mM NaCl/10 mM EDTA) and passed over a polyclonal IgG-Sepharose column (bed volume, 4 ml) and then over the CLI affinity column (1.5 ml). The latter column was washed with 10 ml of Tris-buffered saline containing an additional 1.5 M NaCl and bound proteins were subsequently eluted with 0.2 M glycine/0.5 M NaCl, pH 2.8. The yield of CLI isolated from 20 ml of plasma was about 0.8 mg. The amino-terminal sequence of 10 μg of affinity-purified CLI was determined with an Applied Biosystems 470A/120A gas-phase protein sequencer/phenylthiohydantoin amino acid analyzer system. Complement proteins C5b–6, C7, C8, and C9 were prepared according to standard procedures (1). SC5b–9 complexes were generated by inulin-initiated activation of complement in total human serum and purified as described (14). SDS/

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CLI, cytolysis inhibitor; SGP-2, sulfated glycoprotein 2; C5b–9, complex of complement components C5b, C6, C7, C8, and C9; SC5b–9, soluble (fluid-phase) C5b–9.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25915).

PAGE followed by immunoblotting was performed as described (16, 17).

Quantitation of CLI in Serum and Seminal Plasma. CLI concentrations in normal serum and seminal plasma were determined semiquantitatively by immunoblotting (17). Various dilutions of the samples (kindly provided by M. Germond and A. P. Senn, Centre Hospitalier Universitaire Vaudois, Lausanne) and dilutions of purified CLI as a standard were blotted onto nitrocellulose filters after SDS/PAGE. Incubation with the anti-CLI monoclonal antibody II-F4 (1:1000 dilution of ascites fluid) was performed overnight. The concentration of purified CLI was determined by the Coomassie brilliant blue G-250 binding assay using bovine serum albumin as a standard (Bio-Rad).

cDNA Cloning. A human liver cDNA library constructed in the pGEM-4 vector (18) was screened with the [γ-^{32}P]dATP-labeled oligomers, 5′-CCC-TAT-GAG-CCC-CTG-AAC-TTC-CAC-GCC-ATG-TTC-CAG-CCC-TTC-CTG-GAG-ATG-3′ and 5′-GAC-AAT-GAG-CTG-CAG-GAG-ATG-TCC-AAC-CAG-GG-3′, which were deduced from the partial amino acid sequences Pro-Tyr-Glu-Pro-Leu-Asn-Phe-His-Ala-Met-Phe-Gln-Pro-Phe-Leu-Glu-Met and Asp-Asn-Glu-Leu-Gln-Glu-Met-Ser-Asn-Gln-Gly, respectively (19), according to the method of Lathe (20). The cDNA of CLI was sequenced in the M13 mp8 vector by using the shotgun sequencing strategy and the Sequenase sequencing protocol (United States Biochemical).

Southern Blot Analysis. Total genomic DNA was extracted from spleen of Kfm Wistar rats and from human peripheral blood lymphocytes by standard procedures (21). Restriction fragments of 5 μg of total DNA were size-fractionated by electrophoresis in 0.8% agarose gel and vacuum-blotted (LKB vacuum blotting system) onto GeneScreen*Plus* membranes (DuPont Company) according to the manufacturers' instructions. Southern blot hybridization of total genomic DNA from human and rat species was performed with the random-primed, [α-^{32}P]dATP-labeled cDNA of CLI in 0.9 M NaCl/0.09 M sodium citrate, pH 7.0/0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% SDS containing sheared herring sperm DNA (50 μg/ml) at 58°C overnight. The filters were washed in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% SDS at 58°C for 2 hr and exposed to x-ray film for 2 days (human DNA filter) or 6 days (rat DNA filter) at −75°C.

Functional Assays. Hemolytic tests were performed by diluting C5b–6 to a concentration causing 80% lysis of erythrocytes. Sheep erythrocytes (10^9 cells per ml) in 30 μl of 10 mM Veronal (barbital), pH 7.4/142 mM NaCl/0.1% gelatin/10 mM EDTA were mixed with purified C5b–6 and incubated for 10 min at 20°C. C7 (final concentration, 1 μg/ml) C8 (0.2 μg/ml), and C9 (1 μg/ml), which all had been preincubated with different amounts of CLI for 5 min at 20°C, were subsequently added in a total volume of 30 μl. Alternatively, C5b–7 was preformed on erythrocytes by incubation with the respective components at 37°C for 20 min and CLI, C8, and C9 were added thereafter. After 30 min of incubation at 37°C, the red blood cells were spun down at 1500 rpm for 2 min and the hemoglobin released into the supernatant was measured at 412 nm in a Gilford spectrophotometer. The final concentrations of C5b–6, C7, C8, and C9 were kept constant in all experiments.

RESULTS

Purification and Biochemical Characterization of CLI. The monoclonal antibody II-F4 identified an unknown protein (15), which we now call CLI, in human serum and in the SC5b–9 complex. CLI was purified to homogeneity from human EDTA-treated plasma by affinity chromatography. CLI had the expected molecular mass of 70 kDa under nonreducing conditions and migrated as a heterodisperse band at 35 kDa after reduction (Fig. 1). Amino acid sequence analysis of CLI showed that it consisted of two nonidentical subunits, since each cycle of Edman degradation yielded two different amino acid residues (underlined in Fig. 2 *Upper*). Literature studies revealed that the sequenced residues agreed completely with the partial protein sequences described for each individual subunit of a constituent of the SC5b–9 complex tentatively designated SP-40,40 (19).

Nucleotide and Amino Acid Sequence of CLI. To learn more about the biochemical properties of the SC5b–9-associated CLI, we isolated cDNA clones from a human liver library by using two long oligodeoxynucleotides as probes. One 1.7-kilobase (kb) cDNA clone, identified by its binding to both oligonucleotides, was sequenced. The nucleotide sequence and open reading frame of the 1651-base-pair insert are illustrated in Fig. 2 *Upper*. According to the criteria of Kozak (22), translation of the mRNA transcript starts at position 199 to give a protein of 445 amino acid residues, of which the first 21 amino acids represent a typical signal peptide (23). The derived protein sequence includes both subunits of mature CLI, showing that the two disulfide-linked, nonidentical 35-kDa subunits are generated by proteolytic cleavage of a single-chain precursor between Arg-205 and Ser-206. Each subunit contains three asparagine-linked glycosylation sites (residues 64, 81, 123, 269, 332, and 352) and five closely clustered cysteines, one of which is involved in the disulfide linkage between the amino-terminal and carboxyl-terminal subunits. The two cysteine-containing regions (residues 80–107 and 263–291) do not show sequence homology to each other.

The deduced amino acid sequence of CLI was compared with sequences in the EMBL (release 16.0), GenBank (release 57.0), and NBRF (release 17.0) data bases by using FASTA and TFASTA of the Pearson and Lipman program package (24). An overall 75.6% amino acid sequence homology to rat SGP-2, a major secretion product of rat Sertoli cells (25, 26), was found. In addition, a similar high degree of sequence homology was apparent between CLI and ram rete testis clusterin, for which more than 20 amino-terminal residues of both subunits have been determined (27).

Human CLI and Rat SGP-2 Are Orthologous Gene Products. To evaluate whether SGP-2 and CLI are orthologous proteins in the human and rat species, we performed Southern blot analyses of total genomic DNA of humans and rats and immunoblot analyses with human blood serum and human seminal plasma (Fig. 3). The nearly full-length CLI cDNA, which contains one *Bgl* II site but no *Bam*HI site, detected one *Bam*HI fragment and two *Bgl* II fragments in the

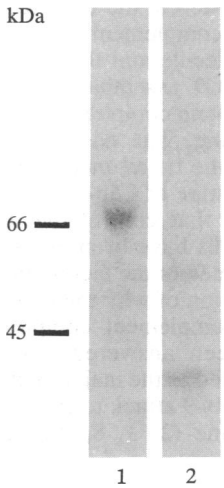

FIG. 1. Affinity-purified CLI (2 μg) in nonreduced (lane 1) and reduced (lane 2) form, analyzed by SDS/PAGE.

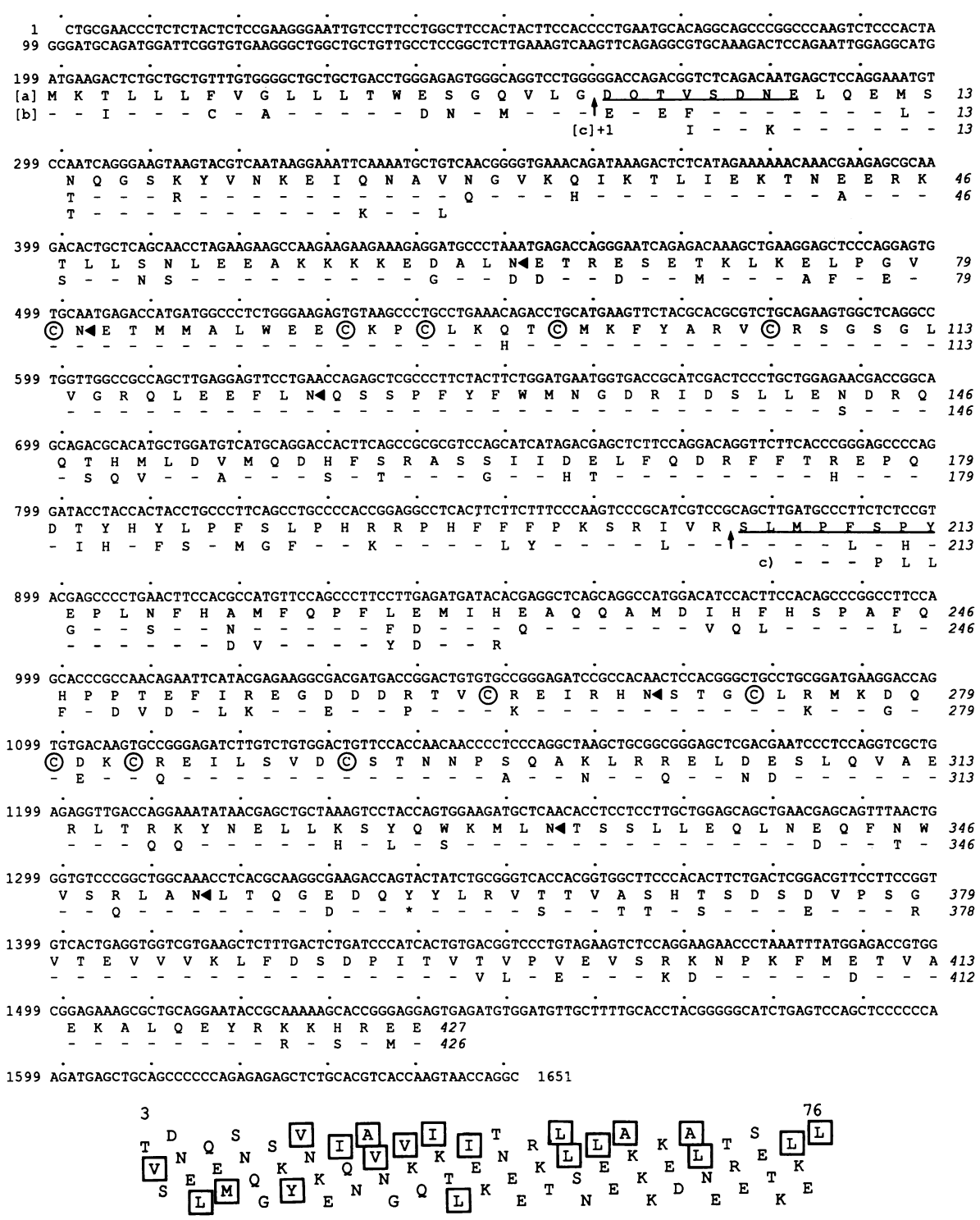

FIG. 2. (*Upper*) Nucleotide and derived amino acid sequences of the human CLI. The deduced amino acid sequence of CLI [a] is shown in single-letter code beneath the cDNA sequence, together with the protein sequence of rat Sertoli cell-derived sulfated glycoprotein 2 (SGP-2) [b] and the partial amino-terminal sequence of sheep clusterin [c]. The underlined amino acid residues were determined by amino acid sequencing of affinity-purified CLI. Amino acid residues −21 to −1 represent the signal peptide; residues 1–205 encode the α subunit, and residues 206–427 the β subunit, of the mature protein. The respective boundaries are marked by vertical arrows. The only gap in the aligned rat SGP-2 sequence is indicated by a star. The six predicted asparagine-linked glycosylation sites are indicated by filled triangles. The two subunits have five cysteines apiece (circled) and are linked by a disulfide bond. (*Lower*) α-Helical depiction of the amino-terminal residues 3–76, which show a high potential to form a dimeric coiled-coil structure. Nonpolar residues (A, V, M, I, L, Y) forming the hydrophobic interface between two amphipathic strands are boxed.

human genome. Digestion of rat genomic DNA with *Bgl* II and *Eco*RI revealed a unique fragment to which the human cDNA probe cross-hybridized (Fig. 3*a*). Furthermore, the anti-CLI monoclonal antibody detected a protein of identical size in human serum and in the SC5b–9 complex and a double band in seminal plasma (Fig. 3*b*). The two molecular weight forms of CLI in seminal plasma are probably due to variation in the carbohydrate structures. We conclude that CLI and SGP-2 (and probably sheep clusterin as well) are encoded by orthologous single-copy genes.

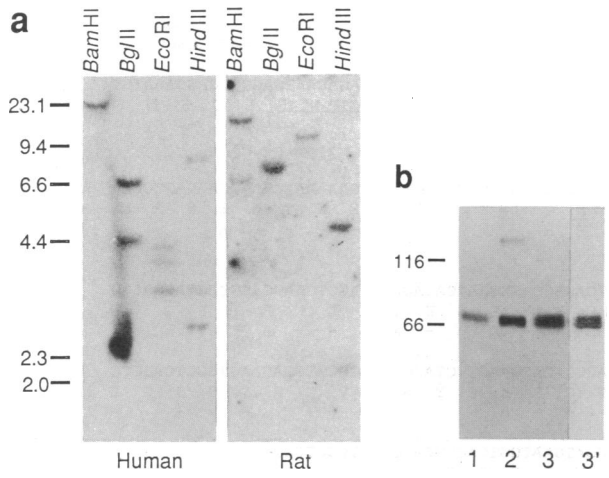

FIG. 3. Identity between human CLI and rat SGP-2. (*a*) Southern blot analysis of human (*Left*) and rat (*Right*) genomic DNA, showing that the CLI of human and rat species is encoded by a single-copy gene. Note that one *Bgl* II site and one *Eco*RI site are present in the cDNA sequence of CLI. Markers at left are in kilobases. (*b*) Immunochemical identification of CLI in human blood serum, in SC5b–9 complex, and in human seminal plasma. Human blood serum (2 μl) (lane 1), SC5b–9 complexes (1 μg) (lane 2), and seminal plasma (0.4 μl) (lane 3 and 3′) were subjected to SDS/PAGE under nonreducing conditions and analyzed by immunoblotting using a mouse monoclonal antibody against CLI. A second, unidentified band (≈140 kDa) was reproducibly stained in the SC5b–9 complexes. Lanes 3 and 3′ both show immunostained CLI of seminal plasma, but the immunoreactive band in lane 3′ is better discernible as a doublet. Markers at left are in kilodaltons.

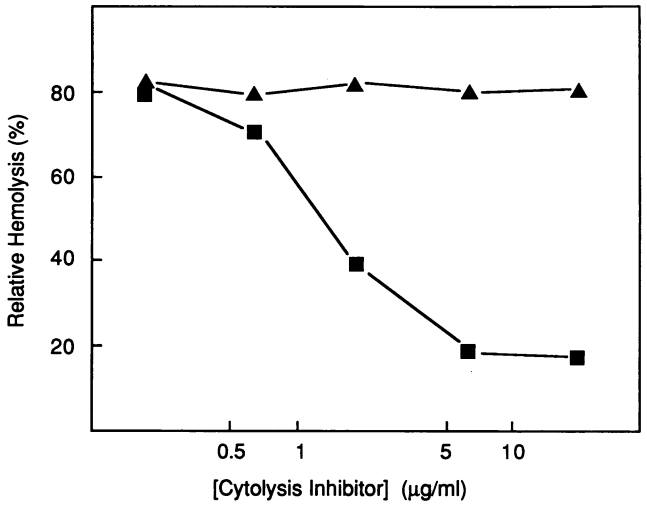

FIG. 4. Anticytolytic action of purified CLI on C5b–6-initiated lysis of sheep erythrocytes in the presence of purified C5b–6, C7, C8, and C9. CLI either was added together with C8 and C9 to C5b–7-bearing erythrocytes (▲) or was added together with C7, C8, and C9 to erythrocytes that had been preincubated with C5b–6 (■). Hemolytic activity (ordinate) is given as the fraction of maximal hemolysis.

The concentrations of human CLI as determined by immunoblotting were studied in 30 healthy men. The blood serum concentration varied between 50 and 100 μg/ml, in accordance with previous data (19). The concentrations in seminal plasma, however, were ≈5 times higher (250–500 μg/ml) than in blood serum.

CLI Inhibits Complement-Mediated Hemolysis. Since CLI is incorporated into the SC5b–9 complex (Fig. 3*b*), resembling S-protein/vitronectin in that respect (19), we reasoned that this protein may play an important inhibitory role in the cytolytic action of terminal complement components. Therefore, we studied the effect of purified CLI on complement-mediated cytolysis. When the cytolytic complement complex C5b–9 was generated from purified components C5b–6, C7, C8, and C9 in the presence of CLI (Fig. 4), the efficacy of hemolysis was suppressed in a concentration-dependent manner. Half-maximal inhibition was observed at a concentration of 1.5 μg/ml, which is below the physiological concentrations of CLI in human serum. No inhibition was observed when C5b–7–erythrocyte intermediates were preformed before CLI addition, suggesting that CLI most likely interfered with membrane binding of nascent C5b–7.

DISCUSSION

The soluble terminal complement complex assembled in inulin-activated serum shows striking compositional differences to the cytolytically active, membrane-bound C5b–9 complex. Besides S-protein (28) and antithrombin III (12), one additional protein has been identified in the SC5b–9 complex in earlier studies (19, 29). Two-dimensional SDS/PAGE gels of the SC5b–9 complex under nonreducing and reducing conditions revealed that the fuzzy protein band that was supposed to consist completely of S-protein migrated in three distinct bands under reducing conditions in the second dimension. The two faint bands at about 75 kDa and 65 kDa corresponded to the known plasma forms of S-protein (14), and the major band at about 40 kDa represented the two subunits of reduced CLI (19), and not a breakdown product of S-protein as was suggested previously (29). As judged from the amount of Coomassie blue-stained CLI (29, unpublished data), CLI appears to be more abundant than the S-protein in the soluble terminal complement complex.

Inhibition of complement-mediated cytolysis was originally explained by the competition of hydrophobic or amphipathic plasma proteins for the hydrophobic, membrane-binding site of nascent C5b–7 complexes. However, the structure of the S-protein did not display any properties to function as the hydrophobic membrane equivalent of the membrane-inserted C5b–9 complex. Recent experimental studies strongly suggested that ionic interactions between the negatively charged "class A" cysteine-rich domain of terminal complement proteins and the heparin-binding region of S-protein trigger the binding of S-protein to nascent terminal complement complexes (30).

In contrast to the S-protein, the amino acid sequence of CLI contains a structural domain at the amino terminus (Fig. 2 *Lower*) that appears to be suitable to interact with the hydrophobic, membrane-inserting domains of nascent terminal complement complexes. Comparing the CLI amino acid sequence with other proteins of the sequence data bases, we found a significant structural similarity between the amino-terminal 76 residues and several other proteins forming long α-helical coiled-coil structures (e.g., heavy chain of myosin, desmin). The structural features characteristic of rodlike proteins (31), especially the typical heptapeptide repeat pattern (a-b-c-d-e-f-g), are present between residues 3 and 76 of CLI (Fig. 2 *Lower*). Many of the amino acid residues at positions a and d in human and rat CLI are apolar and could form the hydrophobic interface between two α-helices. Prolines, which prevent helix formation, and tryptophans, which are rarely found in coiled-coil regions, are absent from the human, rat, and sheep amino-terminal domain (residues 1–76) of CLI. Secondary-structure predictions for rat and human CLI also show a high α-helical potential of the amino-terminal segment. Our hypothesis is supported by the known ability of CLI to self-assemble into noncovalently linked homodimers of ≈160 kDa (19, 32). Formation of CLI dimers may be mediated by the interaction of two amino-terminal

amphipathic α-helices that adopt a two-stranded rodlike coiled-coil structure by hydrophobic helix–helix interactions.

The physiological functions of CLI in blood plasma (SP-40,40; clusterin) and the Sertoli cell-derived CLI in the reproductive tract (clusterin, SGP-2) could not be clarified in the past. Testicular clusterin is able to aggregate cells *in vitro*, in contrast to serum clusterin. This functional difference was shown to result from testes-specific glycosylation of the Sertoli cell-derived clusterin (27). Highly speculative ideas about the function of SGP-2 have also been presented in the case of rat SGP-2 (26).

From our functional studies on the inhibition of complement-mediated cytolysis we conclude that the plasma glycoprotein CLI is a potent specific inhibitor of nascent terminal complement complexes. Since CLI occurs at about 5-fold higher concentrations in human seminal plasma and at about 10-fold higher concentrations in cauda epididymal fluid of the ram (27) as compared to human and sheep blood serum, respectively, and since it is present on the surface of sperm and epithelial cells (33, 34), we suggest that CLI exerts an anticytolytic, protective function in the reproductive system as well. In particular, CLI may prevent complement-mediated immobilization of sperm cells in the reproductive tract of females during fertilization. The strong anti-complement activity previously observed in seminal plasma (35) may be at least in part due to the same protein.

Note Added in Proof. After this manuscript had been submitted, the cDNA sequence of SP-40,40 was published (36). The coding region of the CLI cDNA sequence is identical with that of SP-40,40 except for a synonymous T versus C substitution at position 1218 (Fig. 2 *Upper*).

We thank S. Schaefer for outstanding technical assistance and Z. Freiwald for help in preparing the figures.

1. Müller-Eberhard, H. J. (1986) *Annu. Rev. Immunol.* **4,** 503–528.
2. Müller-Eberhard, H. J. (1988) *Annu. Rev. Biochem.* **57,** 321–347.
3. Tschopp, J. & Jongeneel, C. V. (1988) *Biochemistry* **27,** 2641–2646.
4. Bhakdi, S. & Tranum-Jensen, J. (1987) *Rev. Physiol. Biochem. Pharmacol.* **107,** 147–223.
5. Schönermark, S., Rauterberg, E. W., Shin, M. L., Löke, S., Roelcke, D. & Hänsch, G. M. (1986) *J. Immunol.* **136,** 1772–1776.
6. Zalman, L. S., Wood, L. M. & Müller-Eberhard, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6975–6979.
7. Hänsch, G. M., Weller, P. F. & Nicholson-Weller, A. (1988) *Blood* **72,** 1089–1092.
8. Dahlbäck, B. & Podack, E. R. (1985) *Biochemistry* **25,** 2368–2374.
9. Jenne, D. E. & Stanley, K. K. (1985) *EMBO J.* **4,** 3153–3157.
10. Rosenfeld, S. I., Packman, C. H. & Leddy, J. P. (1983) *J. Clin. Invest.* **71,** 795–808.
11. Nemerow, G. R., Yamamoto, K.-I. & Lint, T. F. (1979) *J. Immunol.* **123,** 1245–1252.
12. Kolb, W. P. & Kolb, L. M. (1983) *Biochemistry* **22,** 496–504.
13. Sassi, F., Hugo, F., Muhly, M., Khaled, A., Rachid, M. S. B. & Bhakdi, S. (1987) *Mol. Immunol.* **24,** 543–548.
14. Jenne, D., Hugo, F. & Bhakdi, S. (1985) *Biosci. Rep.* **5,** 343–352.
15. Bhakdi, S., Jenne, D. & Hugo, F. (1985) *J. Immunol. Methods* **80,** 25–32.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4355.
18. Haefliger, J.-A., Jenne, D., Stanley, K. K. & Tschopp, J. (1987) *Biochem. Biophys. Res. Commun.* **149,** 750–754.
19. Murphy, B. F., Kirszbaum, L., Walker, I. D. & d'Apice, A. J. F. (1988) *J. Clin. Invest.* **81,** 1858–1864.
20. Lathe, R. (1985) *J. Mol. Biol.* **183,** 1–12.
21. Herrmann, B. G. & Frischauf, A.-M. (1987) *Methods Enzymol.* **152,** 180–183.
22. Kozak, M. (1986) *Cell* **44,** 283–292.
23. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
24. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
25. Griswold, M. D., Roberts, K. & Bishop, P. (1986) *Biochemistry* **25,** 7265–7270.
26. Collard, M. W. & Griswold, M. D. (1987) *Biochemistry* **26,** 3297–3303.
27. Chen, C. Y., Mathur, P. P. & Grima, J. (1988) *Biochemistry* **27,** 4079–4088.
28. Kolb, W. P. & Müller-Eberhard, H.-J. (1975) *J. Exp. Med.* **141,** 724–735.
29. Bhakdi, S. & Tranum-Jensen, J. (1982) *Mol. Immunol.* **19,** 1167–1177.
30. Tschopp, J., Masson, D., Schäfer, S., Peitsch, M. & Preissner, K. T. (1988) *Biochemistry* **27,** 4103–4109.
31. Parry, D. A. D. (1982) *Biosci. Rep.* **2,** 1017–1024.
32. Blaschuk, O., Burdzy, K. & Fritz, I. B. (1983) *J. Biol. Chem.* **258,** 7714–7720.
33. Tung, P. S. & Fritz, I. B. (1985) *Biol. Reprod.* **33,** 177–186.
34. Sylvester, S. R., Skinner, M. K. & Griswold, M. D. (1984) *Biol. Reprod.* **31,** 1087–1101.
35. Tarter, T. H. & Alexander, N. J. (1984) *Am. J. Reprod. Immunol.* **6,** 28–32.
36. Kirszbaum, L., Sharpe, J. A., Murphy, B., d'Apice, A. J. F., Classon, B., Hudson, P. & Walker, I. A. (1989) *EMBO J.* **8,** 711–718.