Protein SIC, a Novel Extracellular Protein of Streptococcus pyogenes Interfering with Complement Function*

(Received for publication, August 16, 1995)

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The human pathogen Streptococcus pyogenes possesses a chromosomal region, the mga regulator, that contains co-regulated genes important to the virulence of these bacteria. A novel gene located in the mga regulator of a S. pyogenes strain of serotype M1 was cloned and sequenced. It translates into a protein of 305 amino acid residues, including a signal sequence of 32 amino acids and a central region consisting of three tandem repeats. The sequence represents a novel structure with no significant homology to any previously published sequence. The protein was purified from the streptococcal culture media where it is present in substantial amounts. Affinity chromatography of human plasma on Sepharose coupled with the protein specifically adsorbed two plasma proteins which were identified as clusterin and histidine-rich glycoprotein (HRG). The interactions between the streptococcal protein and the plasma proteins were further characterized using purified clusterin and HRG. Inhibition experiments indicated that they have affinity for overlapping or closely located sites in the streptococcal protein. Both clusterin and HRG are regulators of the membrane attack complex (C5b-C9) of complement. When the streptococcal protein was added to serum, complement-mediated lysis of sensitized sheep erythrocytes and guinea pig erythrocytes was inhibited. In addition, the streptococcal protein was incorporated into C5b-C9 in serum, indicating the location of its action. The name, protein SIC, streptococcal inhibitor of complement-mediated lysis, is therefore suggested for this novel protein. The occurrence of protein SIC and its gene was investigated in a collection of S. pyogenes strains comprising 55 different M serotypes. Only M1 and M57 strains were positive in this screening, indicating that protein SIC could be a virulence determinant. Thus, during recent years, the M1 serotype has been connected with a number of acute supplicative infections such as erysipelas, necrotizing fasciitis, and pharyngitis. These Gram-positive bacteria also cause a serious toxic shock-like syndrome, whereas glomerulonephritis and rheumatic fever are serious poststreptococcal sequelae. To elude the host defense and establish an infection, S. pyogenes has developed multiple molecular mechanisms. Some of these are dependent on genes of the mga regulator. The genes of this regulator are under the control of a positive regulator gene, previously called mga (Capron and Scott, 1987) or virR (Simpson et al., 1990). According to a recent agreement, this regulator should now be called mga, multigene regulator of group A streptococcus.

Based on structural variations in the antiphagocytic M protein (for references, see Fischetti (1989) and Kehoe (1994)), S. pyogenes can be divided into more than 80 different serotypes. Since the late 1980s unusually severe S. pyogenes infections have been reported worldwide, infections which have predominantly been associated with the M1 serotype (for references, see Martin and Single (1993) and Musser et al. (1993)). This serotype is also connected with glomerulonephritis and rheumatic fever. The strain studied here, AP1, is of the M1 serotype. In AP1, the regulatory gene mga is followed by emm1, the gene for M1 protein. Immediately downstream of emm1 is sph (Åkesson et al., 1994), the gene encoding an IgGFc-binding M protein-related molecule called protein H (Gomi et al., 1990; Åkesson et al., 1990). Located adjacent to sph is a previously uncharacterized gene. This gene and its product are the subject of the present report. The data described imply that this extracellular protein, in the following referred to as protein SIC, plays a role in S. pyogenes pathogenicity and virulence through previously unknown molecular mechanisms.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Bacteriophage, and Plasmids—Protein SIC and the sic gene were isolated from S. pyogenes strain AP1 (40/58) of the M1 serotype from the Institute of Hygiene and Epidemiology, Prague, Czech Republic. Forty-eight additional strains of different M serotypes were also obtained from this institute. These strains have been described (Ben Nasr et al., 1995). S. pyogenes strains of serotypes M3, M33, M42, M52, M61, and M67 were kindly provided by Dr. U. Sjöbring, Lund University, and a collection of 35 M1 strains isolated in Sweden 1980–89 were kindly provided by Dr. Stig Holm, Umeå University. One S. pyogenes strain of serotype M1 and two of type M57 were from the late Dr. L. W. Wannamaker. The sic gene was cloned from a λEMBL3 clone described previously (Åkesson et al., 1994). Plasmid vectors pUC18/19 (Yanisch-Perron et al., 1985) and pK18/19 (Pridmore, 1987) were used in subcloning experiments. PCR products were cloned into the inducible secretion vector pHDR389 (Dalbge et al., 1989). The Esch-
erichia coli strain JM109 was used as host for recombinant plasmids. Streptococci were grown in Todd–Hewitt broth supplemented with 0.2% yeast extract and E. coli in LB broth.

Proteins and Antiserum—Recombinant M1 protein, protein PAB, and streptococcal cysteine proteinase (SCP) were purified as described previously (Åkesson et al., 1994; de Château and Björck, 1994; Berge and Björck, 1995). Streptococcal hybridoma serum (HSA) was from Sigma. The monoclonal antibody MCAe11 directed against a neoglycophosphatidyl C9 within the C5b–C9 membrane attack complex (MAC) of complement (Mollnes et al., 1985b) was kindly provided by Dr. T. E. Mollnes, The National Hospital, Oslo, Norway. Antiserum against purified streptococcal protein SIC and SCP were raised in rabbits. Sheep anti-human histidine-rich glycoprotein (HRG) was a kind gift from Dr. William T. Morgan, University of Missouri, Kansas City, MO. Peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) and donkey anti-sheep IgG (ICN) were used as secondary antibodies.

Purification of Protein SIC—Recombinant protein SIC was purified by growing AP1 bacteria to midlog phase (A600 = 0.5) and precipitating the culture supernatant with 30% ammonium sulfate. The resulting precipitate was resuspended to 1/100 of the starting volume with 20 mM Tris, pH 7.5, and dialyzed against the same buffer. This material was applied to a Mono Q column on a fast protein liquid chromatography system (Pharmacia, Uppsala, Sweden). Using a linear NaCl gradient, a single sharp peak was eluted at 0.3 M NaCl. The material was applied to a 5 ml column, concentrated, and subjected to gel filtration chromatography on a Superose 12 column (Pharmacia) in PBSA (0.03 mM phosphate, 0.12 M NaCl, 0.02% NaN3, pH 7.2). Fractions containing the suspected protein SIC were pooled, and the identity of the protein was confirmed by NH2-terminal amino acid sequencing and later by Western blotting using antiserum against protein SIC. The yield was 0.5–2 mg of purified protein SIC per liter of culture. Recombinant protein SIC was purified from an E. coli strain JM109 harboring the sic gene in pHD389. The strain was grown to midlog phase at 30°C before inducing protein expression by adding isopropyl L-thio-D-galactoside (IPTG) to a final concentration of 1.0 mM. Western blotting with antiserum against protein SIC showed that a single band of 80 kDa was detected by SDS-PAGE sample buffer, and subjected to Western blot analysis using rabbit antiserum against protein SIC.

Purification of Clusterin and HRG—For the purification of clusterin and HRG, concentrated fresh human plasma supplemented with 1 mg benzamidine and 0.4 mg phenylmethylsulfonyl fluoride was used. Clusterin was purified by a method modified from a previous study (Wilson and Easterbrook-Smith, 1992). Thus human plasma was differentially precipitated with polyethylene glycol (12–23%, w/v). The precipitate was then passed over an IgG-Sepharose column (Pharmacia Biotech Inc, Piscataway, NJ). The column was washed with 1 M NaCl, and eluted with 0.1 M glycine-HCl, pH 2.0. The material was dialyzed against 20 mM Tris, pH 7.5, and subjected to a Mono Q column. Using a linear NaCl gradient, a protein peak eluted at 0.3 M NaCl was found to contain a single band of 80 kDa when analyzed by SDS-PAGE under nonreducing conditions. The material gave rise to a single band of 35 kDa when run under reducing conditions in the presence of 5% β-mercaptoethanol. The bands were identified as clusterin in Western blot experiments using anti-clusterin antibodies. HRG was purified using the protocol of Saigo et al. (1989). Briefly, plasma was absorbed with proteins against protein SIC, followed by a washing step. Substrate solution, 0.1% (w/v) 2,2′-azinobis(3-ethylbenzthiazolinesulfonate), 0.012% (v/v) H2O2 in 50 mM sodium acetate buffer, pH 5.0.

Affinity Chromatography—In plasma absorption experiments, citrated fresh human plasma was mixed with the protease inhibitors benzamidine and iodoacetic acid to final concentrations of 5 mM each. 5 ml of undiluted plasma was immediately added to 3 mg of protein SIC coupled to Sepharose. After end to end reduction for 4 h at room temperature, the protein SIC-Sepharose was washed extensively with PBS (200 times the column volume), and the proteins bound to the column were eluted with 0.1 M glycine-HCl, pH 2.0. As a control, 5 ml of plasma was absorbed simultaneously with glycine-Sepharose, pH was adjusted to 11. Bound proteins were detected by SDS-PAGE under reducing conditions were boiled in the presence of 5% β-mercaptoethanol. Western blotting was performed by transferring proteins to polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore) as described by Towbin et al. (1979). After blocking, membranes were incubated for 1 h with antiserum against protein SIC (1:200, v/v), HRG (1:200, v/v), or antibodies toward clusterin (1:5000, v/v), or antibodies toward clusterin (1:500, v/v), or antibodies toward clusterin (1:2000, v/v), and binding was detected by a specific rabbit peroxidase-labeled secondary antibody (1:10000 v/v). For visualization of bound antibody, membranes were incubated in 0.2% (w/v) 3-aminio-9-ethylcarbazol, 0.06% (v/v) H2O2 in 50 mM sodium acetate buffer, pH 5.0. The radioactivity in the fractions was measured in a γ-counter.

ELISA—Indirect ELISA (Engvall and Perlmann, 1971) was performed by coating microtiter plates (Maxisorb, Nunc, Denmark) overnight at 4°C with serial dilutions of protein SIC (starting concentration 2 μg/ml). The plates were washed in PBST and incubated with clusterin (5 μg/ml) or HRG (1.25 μg/ml) diluted in PBST containing 1% gelatin (PBSTG). Bound proteins were detected by peroxidase antibodies to clusterin (1:10000 v/v) or anti-HRG (1:2000, v/v), and binding was visualized by a horseradish peroxidase-conjugated secondary antibody against rabbit or sheep IgG (1:5000, v/v). All incubations were done at 37°C for 1 h and followed by a washing step. Substrate solution, 0.1% (w/v) 2,2′-azinobis(3-ethylbenzthiazolinesulfonate), 0.012% (v/v) H2O2 in 100 mM citric acid, 100 mM NaH2PO4, pH 4.5, was added, and the absorbance was determined as described above.

A competitive ELISA was performed using the same procedure specified above except for the following: microtiter plates coated with either clusterin (1.5 μg/ml) or HRG (0.5 μg/ml) were incubated with a mixture of protein SIC (2 μg/ml for binding to clusterin; 1 μg/ml for binding to HRG) and serial dilutions of the competitor protein (starting concentration 20 μg/ml). Bound protein SIC was detected by a specific rabbit antiserum (1:10000 v/v) followed by a secondary antibody toward rabbit IgG (1:3000 v/v) and 2,2′-azinobis(3-ethylbenzthiazolinesulfonate)/H2O2.
Binding of protein SIC to C5b-C9 complexes generated in serum was detected with a capture ELISA modified from Mollnes et al. (1985a). Microtiter plates were coated with 1.0 μg/ml McaE11, a monoclonal antibody against a neoantigen of polymerized C9. Serum diluted 1:1000 in PBSTG was added to the wells followed by serial dilutions of protein SIC or SCP (starting concentrations 10 μg/ml), and the plates were incubated for 3 h at room temperature. Diluted serum was also preincubated with protein SIC or SCP at various concentrations for 3 h, after which generation of C5b-C9 complexes was stopped by addition of EGTA at 10 mM. The incubation mixtures were then transferred to antibody-coated microtiter plates for analysis. Detection of protein SIC and SCP was performed as described above using specific rabbit anti-serum. Results are shown in Fig. 1.

FIG. 1. Nucleotide sequence and deduced amino acid sequence of the sic gene from S. pyogenes strain AP1. The first 188 nucleotides representing the sequence between the protein H gene (sph) and the sic gene and the first 131 nucleotides of the coding sequence have been published (Gomi et al., 1990). In this work, the sequence from nucleotide 7 in the coding sequence was determined. The start of the signal sequence (SS), the NH2-terminal short repeat region (SRR), and the tandem repeats R1–R3 are indicated with arrows. The short repeats (I–V) are marked with bars. Possible –35 and –10 promoter sequences and the ribosomal binding site (RBS) are denoted with dashed lines.

RESULTS

Sequencing of the sic Gene—The previously reported sequence of the protein H gene (sph) revealed the first 138 bp of an open reading frame 188 bp downstream of the stop codon for sph (Gomi et al., 1990). To determine the structure of this possible downstream gene, a phage clone, λ1:4 (Åkesson et al., 1994), harboring both the M1 gene (emm1) and sph was employed for subcloning. A 2.2-kbp SspI/EcoRI fragment of λ1:4 that contained the open reading frame except for the first six nucleotides was cloned into the vector pUC19. Sequencing of this subclone revealed that the entire open reading frame was 915 bp encoding a putative amino acid sequence of 305 residues (Fig. 1). The first 32 amino acid residues represents a typical bacterial signal sequence including a positively charged NH2-terminal region, a hydrophobic core, and a polar cleavage region. The predicted mature protein has 273 amino acid residues and a calculated molecular mass of 30,677 kDa. The most characteristic feature of the sequence is a central repeat region consisting of three tandem repeats of 29, 29, and 21 amino acid residues each (R1–R3), showing 90–95% internal homology. The sequence preceding the R repeats also contains repeats although these are only five or nine amino acids long. In Fig. 1, five different repeats in this short repeat region (SRR) are indicated. They appear only twice each, and, except for repeat IV, they are not in tandem. Instead, some of the repeats are overlapping. The sequence located COOH-terminal of the R repeats is characterized by a high proline content, and, in a short stretch (residue 200–220), the prolines are evenly spaced.
every third or fourth residue. Computer-assisted analysis of the sequence predicted the protein to be highly hydrophilic with a pI of 4.2. A thorough search of the data bases with the entire sequence, or fragments of the sequence including all kinds of repeats, did not reveal any obvious homology either at the nucleotide or the amino acid level.

Location of sic in the mga Regulon of AP1—A further mapping of sic in the mga regulon of the AP1 strain was accomplished by PCR of chromosomal DNA with two sets of primers (Fig. 2). A reaction with the H1 primer corresponding to the sequence of M-like genes encoding the consensus membrane-anchoring motif (Fischetti et al., 1990) and the S2 primer corresponding to the 3′ end of sic generated a fragment of 1.2 kbp. This confirms the intergenic distance of 188 bp between sph and sic as determined by sequencing. The PCR performed with the S3 primer from the 3′ end of sic and the C1 primer corresponding to a part of the signal sequence of the C5a peptidase gene (scpA) generated a 2.2-kbp product. These experiments demonstrate that sic is located 188 bp downstream of sph and approximately 2.1 kbp upstream of scpA (Fig. 2).

Cloning and Expression of Protein SIC in E. coli and Purification of the Protein from Streptococcal Culture Medium—Oligonucleotides were constructed from the sic sequence to generate a PCR fragment corresponding to the mature protein. This fragment was amplified and cloned into the inducible expression vector pHD389. After growing the clone and inducing expression, a periplasmic lysate was examined by SDS-PAGE which showed the overexpression of a 34-kDa protein as compared to the host E. coli strain. Even higher amounts of the 34-kDa product were detected when analyzing the culture medium after precipitation with 80% ammonium sulfate. The protein was purified using precipitation of the culture medium with 30% ammonium sulfate, ion exchange chromatography, and gel filtration (Fig. 3B). The purified product was seen as a single band on SDS-PAGE, and the yield was about 5 mg of purified protein per liter of culture.

Both the presence of a signal sequence and the absence of cell wall anchoring and membrane-spanning sequences in the gene suggested that the protein is secreted by streptococci of the AP1 strain. Consequently, a purification scheme similar to that used for the recombinant protein was applied to the culture medium of streptococcal AP1 bacteria, and a protein with identical migration on SDS-PAGE was obtained (Fig. 3A). The estimated amount of protein SIC in a 1-liter culture was 10–15 mg from which 1–2 mg of purified protein was obtained. NH₂-terminal amino acid analysis of the protein showed that the first five amino acids (ETYTS) were identical with the start of the mature protein as indicated by the nucleotide sequence. Thus, the gene product of sic was shown to be expressed by the AP1 strain as an extracellular protein.

Absorption of Plasma Proteins with Immobilized Protein SIC—Being a secreted product, protein SIC is most likely active outside the bacterial cell. On the streptococcal chromosome, the sic gene is found in a regulon encoding proteins which interact with host molecules, particularly plasma proteins. In order to examine if protein SIC also has affinity for plasma proteins, the molecule was coupled to Sepharose and used to absorb human plasma. After extensive washing, the proteins bound to the protein SIC-Sepharose were eluted with glycine buffer, pH 2.0. The eluted material was lyophilized, resuspended in PBS at 50 times the concentration, and examined on SDS-PAGE. As seen in Fig. 4, two additional bands appeared as compared to the background pattern obtained after absorption with glycine-Sepharose. The bands were electrophoresed onto a PVDF membrane, excised from the membrane, and subjected to NH₂-terminal amino acid sequencing. The band of 80 kDa (labeled I in Fig. 4) showed the presence of two residues in equimolar yields after each sequencing cycle. The two sequences (DQTVSDELLQ and SLMPFSPYEPE) were identical with the NH₂-terminal sequences of the α and β chains of the plasma protein clusterin (Jenne and Tschopp, 1998; Kirsbaum et al., 1989). The band of 70 kDa (labeled II in Fig. 4) had the sequence VSPTDCSAVE, identifying the protein as HRG (Koide et al., 1986). Plasma absorption experiments were performed with recombinant or streptococcal protein SIC coupled to Sepharose, and the same results were obtained.

Further Analysis of the Interaction between Protein SIC and the Plasma Proteins Clusterin and HRG—The binding of protein SIC to clusterin and HRG was now further analyzed. Firstly, clusterin and HRG were purified from human plasma. The observations made with human plasma were then re-examined by affinity chromatography of radiolabeled clusterin and HRG on protein SIC-Sepharose. About 30% of the 125I-clusterin and about 60% of the 125I-HRG were retained after chromatography on a protein SIC-Sepharose column. In contrast, less than 5% of iodine-labeled albumin was eluted from the column (Fig. 5). Additionally, less than 5% of 125I-clusterin or 125I-HRG was bound to columns of M1 protein or HSA.
The interactions were also tested by indirect ELISA in which the binding of clusterin or HRG to protein SIC was shown to be concentration-dependent (Fig. 6). Additionally, competitive ELISAs with clusterin or HRG as the coated proteins and protein SIC as the probe were performed. In these experiments, the binding of protein SIC to clusterin was blocked by HRG although less efficiently than by clusterin itself (Fig. 7A). Similarly, the binding of protein SIC to HRG was inhibited by clusterin (Fig. 7B). The results indicate overlapping or closely located binding sites for the two plasma proteins in protein SIC.

Inhibition of Complement-mediated Hemolytic Activity by Protein SIC—Together with vitronectin, clusterin and HRG bind to the C5b-C9 complex in serum and thereby regulate the cytotoxic action of MAC (Tschopp et al., 1993; Chang et al., 1992). Clusterin partly inhibits hemolysis, while HRG modulates complement function in a biphasic fashion. To assess a possible role for protein SIC in classical pathway-mediated hemolysis, the protein was incubated with diluted human serum for 30 min at 20 °C prior to addition of EA. A dose-dependent inhibition of hemolysis was obtained (Fig. 8A). To exclude nonspecific degradation of complement components during preincubation of serum, an unrelated bacterial protein, the albumin-binding protein PAB of Peptostreptococcus magnus (de Château and Björck, 1994) was used as a negative control. The effect of protein SIC on alternative pathway-mediated hemolysis was examined using GpE as target cells in serum chelated with EGTA and supplemented with Mg²⁺. As shown in Fig. 8B, the effect of protein SIC on hemolysis of GpE was comparable to the effect on hemolysis of EA, consistent with interference at the C5b-C9 level. Similar results were obtained when effects of protein SIC were studied in hemolytic gels with EA and GpE as target cells (Fig. 8C). When protein SIC was applied adjacent to wells containing serum, the zone of lysis caused by serum was partly eclipsed. In contrast, protein PAB had no inhibitory effect on hemolysis.

Binding of Protein SIC to C5b-C9 Complexes—The interac-
tion between protein SIC and the terminal complement proteins in serum was examined with a capture ELISA. In the first layer, a monoclonal antibody was used that detects an epitope of polymerized C9 that is formed during assembly of the C5b-C9 complex. Incubation of serum gives a high level of such complexes binding to the antibody due to spontaneous in vitro activation of complement (Mollnes et al., 1985a). In Fig. 9, an experiment is shown where serum and protein SIC simultaneously were added to and incubated with the solid-phase antibody. The amount of bound protein SIC was then measured. In contrast to the control protein, SCP, protein SIC is incorporated into the C5b-C9 complex generated in serum. No protein SIC binding was detected in the absence of serum or in serum containing EDTA. Further experiments were carried out to ensure that deposition of complement proteins due to activation by the immunoglobulin-coated microtiter plates (Zwirner et al., 1989) did not influence the results. Thus, serum was preincubated with protein SIC. After 3 h, EDTA was added and the incubation mixtures were transferred to the antibody-coated plates for analysis. The results obtained were virtually identical with those shown in Fig. 9. Distribution of Protein SIC in Strains of S. pyogenes—A collection of 55 group A streptococcal strains of different M types were tested for the presence of the sic gene and the expression of protein SIC. At the DNA level, sic was identified by PCR using primers from the start of the coding sequence and the repeat regions and by performing the reaction under low stringency conditions. The expression of protein SIC was tested by growing the strains to midlogarithmic phase, precipitating the culture media with 30% ammonium sulfate, and examining the precipitate in a Western blot using polyclonal antisera against protein SIC. The strains of M types 1 and 57 were positive both in the PCR and in Western blot experiments. The rest of the strains gave no PCR products and were negative in the Western blot analyses. After the initial screening, 35 addi-
tional M type 1 isolates and two M type 57 strains were tested. All of these strains contained sic and expressed protein SIC. The results suggest that the sic gene is highly restricted among various M serotypes, whereas within these serotypes all isolates have and express the gene.

**DISCUSSION**

Analogous to other proteins encoded by genes under the control of mga, protein SIC contains repeated sequences. However, the repeated sequences in mgα, protein SIC does not have the typical structural features of cell wall proteins in Gram-positive bacteria; i.e. a COOH-terminal region anchored to the cell wall through a hydrophobic membrane-spanning domain and a tail of mostly positively charged amino acid residues. The missing cell wall anchor, the occurrence of a typical signal sequence, and the fact that considerable amounts of protein SIC are found in the growth medium suggest that the molecule is secreted and has extracellular function(s).

Previous work has demonstrated several interactions between components of the complement system and proteins encoded by genes of the mga region. Members of the M protein family (M protein, protein Arp, protein Sir, and protein H) have been reported to bind complement factor H, CD46, and/or the C4b-binding protein (Horstmann et al., 1988; Okada et al., 1995; Thern et al., 1995), two proteins with regulatory functions in the complement system. Furthermore, the C5a peptidase (Wexler et al., 1983), which can be released from the streptococcal cell wall by a cysteine proteinase produced by the bacteria (Berge and Björck, 1995), cleaves the C5-derived fragment C5a and destroys its chemoattractant activity for polymorphonuclear leukocytes (Wexler et al., 1985). In the present study, the specific interactions between protein SIC and the plasma proteins clusterin and HRG directed our attention to the final cytolytic step in the complement cascade. Clusterin is known to inhibit the hemolytic activity of complement by binding to MAC (Tschopp et al., 1993; Tschopp and French, 1994), whereas the influence of HRG on MAC is biphasic, inhibitory, or stimulatory, depending on the experimental conditions (Chang et al., 1992). As demonstrated in the present study, protein SIC was inhibitory to hemolysis in classical pathway as well as alternative pathway systems. Furthermore, protein SIC was shown to be incorporated into C5b-C9 complexes formed in serum. Although other mechanisms may also be considered, the findings suggest that the anticomplementary action of protein SIC is focused on the terminal cytotoxic functions of complement.

The metabolically inert erythrocyte is a very sensitive target for MAC whereas most pathogenic bacteria including streptococci are resistant to complement-mediated cytolysis. However, apart from its cytolytic activity, MAC also has proinflammatory effects by stimulating the production and release of inflammatory mediators such as reactive oxygen metabolites, metabolites of arachidonic acid, and cytokines (for references see Morgan (1989)). Bacterial products affecting the various functions of MAC, directly or indirectly, could therefore influence the host-parasite relationship. The molecular complexity necessary to establish and maintain this relationship makes it difficult to predict the consequences of any isolated interaction. However, in the case of pathogenic and virulent bacteria like S. pyogenes, the balance is disturbed, and there is circumstantial evidence (see below) that protein SIC may contribute to the imbalance of the host-parasite relationship in S. pyogenes infections.

To our knowledge, protein SIC is the first bacterial protein reported to interact with clusterin or HRG. Mycobacterium tuberculosis, however, selectively absorbs clusterin from human plasma, suggesting that this important human pathogen could express surface molecules with affinity for clusterin. As mentioned, glomerulonephritis represents a medically significant sequelae following infections with S. pyogenes. In these cases, certain M serotypes are more common, including the two protein SIC-producing serotypes M1 and M57 (Holm, 1988). In post-streptococcal glomerulonephritis, immunoglobulin deposits are found in the glomeruli. Interestingly, MAC is regarded as a mediator of glomerular injury in immune complex-related disease (Couser et al., 1985), and clusterin was found to be co-localized with MAC in biopsies from glomerulonephritic kidneys (French et al., 1992). Also, plasma depleted of clusterin (to <30%) enhanced proteinuria and deposition of MAC components in perfused kidneys (Saunders et al., 1994). The association of protein SIC to nephritogenic M serotypes, and its binding of clusterin in human plasma makes it interesting to test whether protein SIC can induce kidney damage in an animal model.

Since the late 1980s, a world-wide increase of hyperacute, toxic, and often lethal S. pyogenes infections has attracted public attention also (Nowak, 1994). These systemic infections have been associated particularly with streptococci of the M1 serotype, and the observation that all M1 strains tested, including isolates from Swedish patients with toxic and severe infections (Holm et al., 1992), carry and express the sic gene, supports the notion that protein SIC plays a role in pathogenicity and virulence. The present work and future studies on protein SIC may therefore clarify molecular mechanisms which could be used as targets to prevent and treat S. pyogenes infections.

Acknowledgments—We are grateful to Chun-Li Liu and Kristin Persson for excellent technical assistance.

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