Protein GRAB of *Streptococcus pyogenes* Regulates Proteolysis at the Bacterial Surface by Binding α₂-Macroglobulin*

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In the molecular interplay between pathogenic microorganisms and their host, proteolytic mechanisms are believed to play a crucial role. Here we find that the important human pathogen *Streptococcus pyogenes* (group A *Streptococcus*) expresses a surface protein with high affinity ($K_a = 2.0 \times 10^{9} \text{M}^{-1}$) for α₂-macroglobulin (α₂M), the dominating protease inhibitor of human plasma. The immunoglobulin-binding protein G of group C and G streptococci also contains an α₂M-binding domain and a gene encoding protein GRAB (protein G-related α₂M-binding protein) was identified in the *S. pyogenes* Genome Sequencing data base. The *grg* gene is present in most *S. pyogenes* strains and is well conserved. Protein GRAB has typical features of a surface-attached protein of Gram-positive bacteria. It also contains a region homologous to parts of the α₂M-binding domain of protein G and a variable number of a unique 28-amino acid-long repeat. Using *Escherichia coli*-produced protein GRAB and synthetic GRAB peptides, the α₂M-binding region was mapped to the NH$_2$-terminal part of protein GRAB, which is the region with homology to protein G. An isogenic *S. pyogenes* mutant lacking surface-associated protein GRAB showed no α₂M binding activity and was attenuated in virulence when injected intraperitoneally in mice. Finally, α₂M bound to the bacterial surface via protein GRAB was found to entrap and inhibit the activity of both *S. pyogenes* and host proteinases, thereby protecting important virulence determinants from proteolytic degradation. This regulation of proteolytic activity at the bacterial surface should affect the host-microbe relation during *S. pyogenes* infections.

*Streptococcus pyogenes* is an important human pathogen that causes a variety of diseases such as pharyngitis, impetigo, scarlatina, and erysipelas. More severe infections caused by this organism are necrotizing fasciitis and streptococcal toxic shock-like syndrome. *S. pyogenes* binds several human plasma proteins via its surface proteins. The surface proteins studied in most detail are the M or M-like proteins, which are responsible for the ability of *S. pyogenes* to resist phagocytosis (1, 2).

M proteins bind several human plasma proteins like fibrinogen (3), IgG (4, 5), and regulatory proteins of the complement system (6, 7). The binding activities of M proteins have been proposed to be of importance for the antiphagocytic activity of these proteins (2, 6–9).

Several different streptococcal species also bind α₂M (10–16), which is an abundant homotetrameric plasma protein of 718 kDa best characterized as a proteinase inhibitor (17). During infection proteinases are released both by bacteria and damaged host cells, and tight regulation of proteolytic activity is thus essential. *S. pyogenes* secretes a cysteine proteinase (SCP), which has several important functions and is regarded as a major virulence factor of this bacterium (18–25). There is no described inhibitor of the SCP even though it is known that essentially all proteinases from the four classes (metallo, cysteine, aspartic, and serine proteinases) are inhibited by α₂M (26). Inhibition of proteinases by α₂M is achieved by cleavage of the bait region of α₂M, which induces cleavage of an internal thioester in α₂M (26). The free glutamyl group of the thioester can form amide bonds with lysyl amino groups in the proteinase (27). These events induce conformational changes in the structure of α₂M and results in an entrapment of the proteinase (28). The trapped enzyme remains active against smaller substrates but is sterically hindered to cleave larger ones (26). The proteinase complexed form of α₂M is recognized and cleared by a specific receptor, referred to as low density lipoprotein receptor-related protein, present on hepatocytes, macrophages, and fibroblasts (29). α₂M has also been implicated in immunoregulatory events by direct effects on macrophages, modulation of the effect of growth factors and cytokines, and effects on antigen presentation (30–33). Furthermore α₂M is a major carrier for zinc and cadmium in plasma (34).

The interaction between streptococci and the two forms of α₂M is highly specific. Human pathogenic streptococci (groups A, C, and G) only bind to the native form of α₂M, whereas bovine and equine group C streptococci interact with the proteinase complexed form of α₂M (16). The binding of native α₂M to group C and G streptococci has been attributed to protein G (11, 12), a surface-associated molecule with separate binding sites also for human IgG and human serum albumin (35–37). An α₂M-binding surface protein of 78 kDa from group A streptococci has also been described, but the sequence of this protein is not known (10). In the present work a novel α₂M-binding protein in *S. pyogenes* is identified and characterized. A role is demonstrated for this molecule, called protein GRAB (protein G-related α₂M-binding protein), in the regulation of proteolytic activity at the streptococcal surface. Thus, important bacterial

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§ The abbreviations used are: α₂M, α₂-macroglobulin; SCP, streptococcal cysteine proteinase; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; MBP, maltose-binding protein; aa, amino acid(s).
surface proteins and virulence determinants are protected from proteolytic degradation by α-M bound to protein GRAB.

**Experimental Procedures**

**Bacterial Strains and Growth Conditions**—*S. pyogenes* strains denoted AP are from the Institute of Hygiene and Epidemiology (Prague, Czech Republic). The KTL3 and KTL9 strains are blood isolates, and the KTL6 is a throat isolate from the Finnish Institute for Health. The SF370 strain is the ATCC 700294 strain. For molecular cloning purposes the DHE5 strain of *Echerichia coli* was used. Streptococci were grown in Todd-Hewitt broth (Difco, Detroit, MI) with 0.2% yeast extract (Difco) (THY) in 5% CO2 at 37 °C. *E. coli* were grown in Luria Bertoni broth (10 g of tryptone, Difco, 10 g of NaCl, and 5 g of yeast extract (Difco/filter) supplemented with 2 g glucose/1iter when using the pMal-p2 vector. For growth on Petri dishes 15 g/iter of baco agar (Difco) was added. When *E. coli* contained plasmid either 100 μg/ml ampicillin (Sigma) or 50 μg/ml of kanamycin (Sigma) was added to the medium.

**Proteins and Peptides**—Human α-M was purified from fresh frozen plasma, and wild-type protein G was prepared from group G streptococcus strain GI48 as described (11). The streptococcal cysteine proteinase was purified and tested as described (22). Peptides with sequence from the proteinase GRAB on its surface, a fragment of GRAB on its surface, a fragment of GRAB on its surface, a fragment of GRAB on its surface were washed three times for 10 min using PBS with 0.05% Tween 20 before harvest by centrifugation at 3,800 g for 10 min of plasma in 200 μl of PBS. Following incubation for 15 hr + 15 g agar/l with 150 μg of kanamycin/ml.

**Animal Experiments**—Female NMRI mice weighing approximately 25 g were injected intraperitoneally with washed bacteria from an overnight culture in 1 ml of sterile PBS. The number of bacteria injected was determined by spectrophotometry and verified by colony counting. Mice were observed for 7 days after challenge, and survival was assessed at intervals of roughly 2 hr. Blood samples were drawn from ill mice injected with MR4 and plated on THY plates with and without kanamycin to confirm the presence of the plasmid on the bacterial chromosome. Statistical analysis of survival time was performed with the Wilcoxon rank sum test.

**Northern Blotting**—Total RNA from *S. pyogenes* was purified using a Fastprep® cell disruptor (Savant, Holbrook, NY) as described previously (43). KTL3 or AP1 bacteria were cultured in THY medium either for 12 hr (late stationary phase) or to an A620 of 0.4 (early logarithmic phase), 0.65 (late logarithmic phase), or 0.8 (early stationary phase), before harvest by centrifugation at 8,500 g for 10 min at 4 °C. Pellets were washed twice in water and resuspended by disrupting in 6.0 using FastRNA® kit with glass beads (BIO 101, Vista, CA) according to the manufacturers instructions. For Northern blot experiments, 5 μg of total RNA was separated on 1% agarose in HEPES buffer (0.2 mM Na-HEPES, pH 7.0, 50 mM NaCl, 10 mM EDTA), blotted onto Hybond-N filters (Amersham Pharmacia Biotech), and hybridized with probe, generated by PCR from KTL3 using primers 1 and 5. Probe was purified on a MicroSpin® S-200 HR column (Amersham Pharmacia Biotech) and radiolabeled with [α-32P]dATP using Megaprime® (Amersham Pharmacia Biotech). To estimate the amount of RNA loaded in each well, a probe was constructed from 16 S ribosomal RNA sequence from *S. pyogenes*. This sequence was obtained by homology search between the Streptococcal Genome Sequencing Project sequence data base and 16 S sequence of *Enterococcus faecalis* (accession number X55133). From this sequence two oligonucleotides 5′-TACT GTA ATT TCA AAG GGG-3′ and 5′-TTT AAG AGA TTA GCT TGC CGT-3′ were used to PCR amplify an ~800 base pair fragment from KTL3 DNA, which was labeled as above. Hybridization was performed at 50 °C for 14 hr. After hybridization the membranes were washed in 6× SSC + 0.1% SDS and then in 0.1× SSC + 0.1% SDS and air dried followed by exposure to X-ray film (Amersham Pharmacia Biotech).

**Analyses of Proteolysis**—2 × 109 KTL3 or MR4 cells were incubated with 20 μg of α-M for 40 min and carefully washed with PBS. These bacteria were either incubated with radiolabeled trypsin for 5 min followed by the determination of bound radioactivity as above or with 0.3 μg of unlabeled trypsin, which was allowed to react with surface bound α-M for 5 min. Free trypsin (not in complex with α-M) was removed by adding a 4-fold excess of soybean trypsin inhibitor. Cells were pelleted by centrifugation, and the resulting pellet was washed once in 1 ml of PBS and resuspended in 150 μl of PBS supplemented with 40 μg of chloramphenicol/ml (Roche Molecular Biochemicals). The remaining activity of trypsin in the supernatant and the resuspended pellet was determined using the chromogenic substrate N-benzoyl-L-arginine p-nitroanilide (Sigma) at a final concentration of 0.25 mg/ml by measuring A405 after 3 hr. The obtained value for MR4 was subtracted from that of KTL3 and compared with a standard, where the same assay was run in parallel using purified α-M of known concentration. For protection assays, bacteria were preincubated with α-M or fibrinogen (20 μg) as above and treated with 0.1 μg of trypsin in PBS with chloramphenicol as above for 60 min at 37 °C. Bound fibrinogen was eluted twice with 0.1 M glycine, pH 2.0, and bacteria were diluted 10 times in PBS/PG supplemented with 10 mM benzamidine (Sigma) and chloramphenicol as above. 4 × 10⁶ bacteria were tested for binding of radiolabeled fibrinogen.

Radiolabeled SCP was activated inactivation buffer (1 mM EDTA and 10 mM dithiothreitol in 0.1 M NaAc-HAc, pH 5.0) for 30 min at room temperature. To the activated SCP (4 μg) and either 10 μg of α-M or 0.2 μg of plasma in 20 μl of PBS. Following incubation for 15 min at 37 °C the mixture was subjected to SDS-PAGE using nonreducing conditions followed by autoradiography. 2 × 10⁶ bacteria were preincubated with 10 μg α-M, washed, and incubated with radiolabeled and activated SCP for 15 min. Bacteria were pelleted by centrifugation, and the pellet was washed with 3 ml of PBSAT and recentrifuged. The radioactivity of the pellet was measured, and bound material was released by suspension of the putative cell wall attachment region was generated by PCR from the KTL3 strain using primers 2 and 4. The fragment was cut with *XhoI* and HindIII which exclusively cut within primers 2 and 4, respectively, and cloned into the corresponding site of the streptococcal suicide plasmid pFW13 (30) and electrooporated into *E. coli*. Plasmid was purified, digested, and cloned into the corresponding site of KTL3 plasmid to allow for homologous recombination to occur. Transformsants were plated on THY + 15 g agar/l with 150 μg of kanamycin/ml.
pellet in nonreducing SDS-PAGE sample buffer. Finally the eluate was subjected to SDS-PAGE and autoradiography. Alternatively bacteria were pretreated with 10 μg of αM, washed, and digested with 0.2 μg of activated SCP in 100 μl of PBS (final concentration of dithiothreitol, 0.2 mM) with chloramphenicol as above for 2 h at 37 °C. Bacteria were pelleted and resuspended in PBSAT with 6 mM of iodoacetamide (Sigma), and 4 × 10^6 bacteria were subjected to binding assay with radiolabeled fibrinogen in the same buffer.

**Other Methods**—For precipitation of proteins the sample was incubated with 6% trichloroacetic acid for 30 min on ice followed by centrifugation at 15 000 × g (4 °C for 20 min). Homology searches were performed using available sequences from the Streptococcal Genome Sequencing Project (Department of Chemistry and Biochemistry, the University of Oklahoma, Norman, OK) and at the National Center for Biotechnology Information by using the BLAST network service (Wisconsin package, version 8, Genetics Computer Group, Madison, WI).

**RESULTS**

*Streptococcus pyogenes* Binds Native αM via a Protein G-like Protein—Bacteria from different strains of *S. pyogenes* were harvested in late logarithmic growth phase and tested for their ability to bind radiolabeled native αM (Fig. 1A). The binding ranged from 0 to 76% and differed between strains also within a given M serotype. No strain bound the trypsin complexed form of αM (data not shown). The KTL3 strain of the clinically important M1 serotype, which bound 53% of added αM, was chosen for further studies. The binding of radiolabeled αM to the KTL3 strain was blocked both by nonradioactive αM and by protein G (Fig. 1B). The Scatchard plot for the reaction between αM and KTL3 bacteria (Fig. 1C) suggests two interactions: a high affinity interaction, K_a = 2.0 × 10^6 M^-1 (560 sites/bacterium), and a low affinity interaction, K_a = 5.3 × 10^5 M^-1 (4000 sites/bacterium). Because binding of αM to the KTL3 strain could be competed by protein G, we hypothesized that the αM binding was mediated by a protein G-related protein. Thus, the protein sequence of protein G from strain G148 was used in a tBLASTn search against the Streptococcal Genome Sequencing Project data base (44). A gene coding for a protein showing homology to the αM-binding E domain (11), to the signal sequence, and to the cell wall attachment of protein G, was identified. The protein, named protein GRAB, consisted of 217 amino acids (aa) with a deduced molecular mass of 22.8 kDa. In Fig. 2A a schematic representation of the homology between protein GRAB and protein G is shown. Protein GRAB was found to contain the consensus sequence for Gram-positive surface proteins (LPXTGXX), followed by a stretch of 19 hydrophobic amino acids and a 7-residue-long hydrophilic COOH terminus (Fig. 2B). The first 34 aa of protein GRAB showed homology to the αM-binding E domain (11), to the signal sequence, and to the cell wall attachment of protein G (Fig. 2B). Spacing the regions with homology to protein G, two unique repeated regions of 28 aa were identified in protein GRAB. A BLASTp search revealed that protein GRAB has significant homology to several surface proteins from Gram-positive bacteria. The predicted mature protein GRAB (aa 35–187 in Fig. 2B) is, however, homologous only to protein G (accession numbers X06173, M13825, and X53324) and to an albumin-binding protein from *Streptococcus canis* (accession numbers A44801 and M95520).

**Distribution and Expression of the grab Gene**—The same strains that were used in the screening for αM binding were subjected to PCR using primers hybridizing with *grab*. A PCR product could be generated from all strains except for the AP9 strain, but the size of the product varied between 500 and 850 base pairs (Fig. 3A). Sequencing of the PCR product from four strains revealed that the size polymorphism was due to a variable number of the 28-aa repeats (Fig. 3B). Comparing the sequences from these four strains and the one presented in the Streptococcal Genome Sequencing Project revealed that protein GRAB is highly conserved. Both the COOH and the NH2 termini were close to 100% conserved, whereas the repeated region showed 86% identity between strains (Fig. 3B). The transcription of *grab* was investigated by Northern blotting where total RNA samples from the αM-binding KTL3 strain and the nonbinding AP1 strain were electrophorized, blotted, and probed with a PCR product generated from *grab*. Detectable amounts of *grab* RNA was found in KTL3 but not in AP1 bacteria (Fig. 4). Maximum expression was found in early logarithmic phase, whereas the expression dropped to undetectable amounts in the late stationary phase. The same filters were probed with a probe hybridizing with 16 S ribosomal RNA, which verified that the same amount of RNA had been applied to each well (Fig. 4).

The NH2-terminal Region of Protein GRAB Interacts with
DNA encoding the predicted mature protein GRAB (aa 34–189 in Fig. 2) in the KTL3 strain was PCR cloned into the pMal-p2 vector to produce a fusion protein between MBP and protein GRAB. The fusion protein, MBP-GRAB, was purified by affinity chromatography on an amylose resin, subjected to SDS-PAGE, and blotted to a nitrocellulose filter. The filter was probed with radiolabeled $\alpha_2M$, and both protein G and the MBP-GRAB fusion were found to bind $\alpha_2M$, whereas MBP did not (Fig. 5A). Similarly MBP-GRAB, protein G, and MBP were applied in slots to a nitrocellulose membrane and probed with $\alpha_2M$ and again MBP-GRAB bound $\alpha_2M$, whereas MBP did not (Fig. 5B). Moreover MBP-GRAB, but not MBP, was found to compete for the binding of radiolabeled $\alpha_2M$ to KTL3 bacteria (Fig. 6). Thus, both protein GRAB and protein G can inhibit the binding of $\alpha_2M$ to KTL3 bacteria, indicating that the two proteins interact with the same region in $\alpha_2M$. Finally a peptide covering the extreme NH$_2$ terminus of the mature protein GRAB (aa 34–56 Fig. 2B) inhibited the binding of $\alpha_2M$ to KTL3 bacteria, whereas an overlapping peptide (aa 49–68 in Fig. 2B) did not affect the binding (Fig. 6). The results map the binding
of αM to the NH$_2$-terminal part of protein GRAB.

A S. pyogenes Mutant Devoid of Protein GRAB on Its Surface Does Not Bind αM and Is Attenuated in Virulence—To inactivate $\text{grab}$ a PCR-generated 468-base pair internal fragment (nucleotides 113–580 in Fig. 2B) of $\text{grab}$ lacking the part encoding the cell wall anchoring region was cloned into the pFW13 suicide vector to generate pFW-$\text{grab}$ (Fig. 7A). pFW-$\text{grab}$ was electroporated into KTL3 bacteria for homologous recombination (Fig. 7A), and several kanamycin-resistant transformants were obtained. Using this cloning strategy the
mutant should be devoid of surface bound protein GRAB and instead secrete a truncated form (aa 34–174 in Fig. 2B). One transformant called MR4 was selected, and its ability to bind radiolabeled $\alpha_M$ was completely abolished (Fig. 7A). Moreover, when the supernatants from an overnight culture of MR4 and KTL3 were precipitated with trichloroacetic acid, subjected to SDS-PAGE, blotted to nitrocellulose, and probed with radiolabeled $\alpha_2M$ (Blot). C, NMRI mice were injected intraperitoneally with $10^9$ KTL3 or MR4 bacteria, and mice were followed for 7 days. Mean time to death, with the range in parentheses, was significantly longer in mice injected with MR4 ($p = 0.005$).
Radiolabeled and activated SCP was mixed with either purified $\alpha_2$M or with plasma and subjected to nonreducing SDS-PAGE and autoradiography. Part of the radioactivity appeared as a band with the apparent size of $\alpha_2$M, indicating that a covalent complex had been formed between SCP and $\alpha_2$M (Fig. 9A). Pretreatment of KTL3 and MR4 with $\alpha_2$M resulted in a 2.6-fold increased binding of radiolabeled and activated SCP to KTL3 (2.6 ± 0.57% compared with 6.85 ± 0.92%). Binding of radiolabeled, activated SCP to MR4 bacteria was below 2.5% and was not affected by $\alpha_2$M pretreatment.

When bound material was eluted from the bacteria and subjected to SDS-PAGE and autoradiography, SCP was in complex with $\alpha_2$M in the KTL3, but not in the MR4 material (Fig. 9B). The supernatants were separated on the same gel, and a small proportion of the radioactivity from the $\alpha_2$M pretreated KTL3 bacteria could be seen as band with the apparent size of $\alpha_2$M (data not shown).

SCP can release biologically active fragments of streptococcal surface proteins, one of which is a fibrinogen-binding fragment of the M protein (22). To test whether $\alpha_2$M could inhibit this proteolytic cleavage, KTL3 and MR4 bacteria were harvested at logarithmic growth phase. The bacteria were incubated with $\alpha_2$M, washed, and subjected to SCP digestion. The bacteria were tested for their ability to bind radiolabeled fibrinogen. The results show that $\alpha_2$M pretreatment preserved the fibrinogen binding of the KTL3 strain and not of the MR4 strain (Fig. 9C). The experiments described above demonstrate that $\alpha_2$M in solution, or bound to S. pyogenes via protein GRAB, can trap SCP and thereby protect M protein from SCP cleavage.

**DISCUSSION**

The combined work of many research groups has emphasized the significance of proteolysis in microbial pathogenesis, and the starting point for this study was to investigate the molecular basis for the interaction between S. pyogenes and $\alpha_2$M, a major proteinase inhibitor of human plasma. The finding that wild-type protein G of group C and G streptococci could inhibit this interaction lead to the identification of protein GRAB, a previously unknown $\alpha_2$M-binding surface molecule in S. pyogenes. Protein G is widely known and used as an IgG-binding reagent (35, 36). However, this multifunctional protein also has...
affinity for human serum albumin (37) and \( \alpha_{2}M \) (12). Whereas the interactions with IgG and human serum albumin are mediated by separate repeated domains (45, 46), \( \alpha_{2}M \)-binding is located in the NH\(_2\)-terminal nonrepeated E domain of protein G (11). The homology between the far NH\(_2\)-terminal sequences of protein G and protein GRAB and the results of inhibition experiment performed with synthetic peptides based on the protein GRAB sequence maps the binding of \( \alpha_{2}M \) to the very tip of these streptococcal surface proteins. It is possible that the large and bulky \( \alpha_{2}M \) molecule requires this kind of exposed binding site, which will also allow \( \alpha_{2}M \) to interact simultaneously with more than one protein GRAB molecule. This would increase the affinity of the interaction and could perhaps explain the high affinity interaction between protein GRAB and \( \alpha_{2}M \). The nature of the low affinity interaction is not known, and it is not likely to be of importance, because the amount of \( \alpha_{2}M \) bound to the bacteria corresponds to the number of high affinity binding sites.

The grab gene was found in 11 of 12 tested strains of S. pyogenes, and the NH\(_2\)-terminal A domain of protein GRAB is well conserved. Thus, in the five isolates where the grab sequence is known, the identity was 98% at the amino acid level, suggesting that \( \alpha_{2}M \) binding adds selective advantages to streptococci. This notion is also supported by the similar \( \alpha_{2}M \) binding properties of proteins GRAB and G and by the observation that their 35 NH\(_2\)-terminal amino acid residues show 74% identity. Given the short generation times in bacteria and the fact that the proteins are found in different bacterial species, this represents a remarkably high degree of homology. On the other hand the number of repeats in protein GRAB differs considerably between isolates, and their sequences are less conserved as compared with the A, W, and M domains. The function of these repeats is unknown, but they could play a role as spacers, exposing the \( \alpha_{2}M \)-binding A domain at the bacterial surface.

In the MR4 strain, where the cell wall anchor of protein GRAB had been deleted to give rise to a secreted form of protein GRAB, the binding of \( \alpha_{2}M \) was completely lost. To investigate whether \( \alpha_{2}M \) binding and protein GRAB was involved in the virulence of S. pyogenes, we used a mouse intraperitoneal challenge model. The time from injection to death of the animals was significantly prolonged using MR4 as compared with KTL3 bacteria, suggesting a role for protein GRAB and proteinase inhibition in S. pyogenes virulence. Protein GRAB is the only \( \alpha_{2}M \)-binding surface protein expressed by KTL3 bacteria and the S. pyogenes \( \alpha_{2}M \)-binding protein reported by Chhatwal et al. (10) is larger than protein GRAB (78 kDa as compared with 23 kDa). The mode of interaction between protein GRAB and \( \alpha_{2}M \) is also different from that of the 78-kDa protein and \( \alpha_{2}M \). When bound to the 78-kDa protein, \( \alpha_{2}M \) was reported to be converted to a non-native form (10). This is in contrast to the \( \alpha_{2}M \) bound to KTL3 cells via protein GRAB. In this case \( \alpha_{2}M \) is still active as a proteinase inhibitor.

The cysteine proteinase produced by S. pyogenes was the first prokaryotic cysteine proteinase to be isolated (18). This extracellular enzyme efficiently releases biologically active fragments of S. pyogenes surface proteins, including members of the M protein family and a C5a peptidase (22). In vivo most molecular mechanisms are tightly controlled, suggesting that the proteolytic activity of SCP at the bacterial surface could be regulated by proteinase inhibitors. The observation that kininogens, human plasma proteins, and cysteine proteinase inhibitors, have affinity for M proteins (47) supported this hypothesis. However, instead of being inactivated, SCP was found to cleave kininogens, resulting in the release of the potent proinflammatory peptide bradykinin (24). The demonstration here that \( \alpha_{2}M \) bound to protein GRAB traps and thereby inhibits the cleavage of M proteins by SCP represents a mechanism by which a bacterial proteinase is regulated by a host proteinase inhibitor bound to a bacterial surface protein. Such a mechanism has presumably not been reported.

The anti-phagocytic M protein is very trypsin-sensitive (1). As shown here \( \alpha_{2}M \) bound to protein GRAB protects the M protein from trypsin degradation. This implicates that the presence of \( \alpha_{2}M \), a broad spectrum proteinase inhibitor, at the bacterial surface protects the bacterium and its surface proteins from proteolytic attack. At the site of infection proteinases are actively produced and secreted by neutrophils, whereas damaged cells and tissues passively leak intracellular proteinases. The activity of these enzymes is believed to facilitate spreading of the infection by tissue degradation. Binding of proteinase inhibitors like \( \alpha_{2}M \) and kininogens to bacterial surfaces could therefore, apart from protecting the bacteria from proteolysis, deplete the microenvironment from proteinase inhibitors. Such a mechanism would enhance inflammation and tissue degradation. More recently it has become clear that several bacterial species, including S. pyogenes, survive and multiply within, for instance, epithelial cells (48). It could be speculated that \( \alpha_{2}M \) bound to the bacterial surface via protein GRAB protects the microorganism from intracellular proteinases. Apart from its function as a proteinase inhibitor, a role for \( \alpha_{2}M \) has also been implicated in immune regulation (30–33). Moreover \( \alpha_{2}M \) is an important carrier of zinc and other trace metals in plasma (34), and binding of \( \alpha_{2}M \) to the bacterial surface could be a mean for the bacterium to capture these essential metal ions. If protein GRAB and its \( \alpha_{2}M \) binding activity is connected with important biological functions in S. pyogenes, this would explain the highly conserved NH\(_2\)-terminal sequence in the A domain and make protein GRAB a potentially interesting vaccine target.

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