Protein G–related \( \alpha_2 \)-macroglobulin–binding (GRAB) protein is a cell wall–attached determinant of group A streptococcus (GAS) that interacts with the human protease inhibitor \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)-M). Of 86 clinical isolates tested, 23% could bind \( \alpha_2 \)-M. However, all strains tested contained the \textit{grab} gene. High levels of anti-GRAB antibodies were found in the serum of convalescent GAS-infected patients, a finding that indicates that this protein is expressed during the infection process. Among the \( \alpha_2 \)-M–binding strains, 80% were skin isolates, and 20% were throat isolates, findings that suggest that the skin environment is a preferential site for expression of \( \alpha_2 \)-M–binding activity. To test this possibility, we determined the role of GRAB in a mouse model of GAS skin infection. The wild-type strain KTL3, which interacts with \( \alpha_2 \)-M, showed high virulence. The isogenic mutant of KTL3, MR4, devoid of surface-bound GRAB, was attenuated in virulence, compared with the wild-type strain. Thus, mice infected with MR4 survived longer, developed smaller skin lesions, and exhibited lower levels of bacterial dissemination than did those infected with KTL3. These results emphasize the role of GRAB as a virulence factor of GAS.

Group A streptococci (GAS) are important human pathogens able to cause a wide spectrum of clinical manifestations, ranging from mild, selflimiting infections, such as pharyngitis and pyoderma, to more severe invasive diseases, such as necrotizing fascitis and streptococcal toxic shock–like syndrome [1, 2]. Since the 1980s, a resurgence of severe, invasive GAS infections has been observed, with a particularly high number of necrotizing fascitis cases [3–5]. Although the reasons for the resurgence of severe GAS infections are not yet clear, the emergence of GAS strains with increased virulence could be one of the factors [6, 7]. Severe soft-tissue infections are characterized by intense tissue necrosis that rapidly spreads from the original site of infection [3]. For this purpose, GAS display a complex array of virulence factors directed at facilitation of bacterial spread, by the degradation of extracellular matrix. Thus, in addition to being able to produce proteases [8–14], DNase [15], and hyaluronidase [16], GAS is able to interact directly with human components, such as plasminogen, and is able to acquire plasminlike enzymatic activity [17–21]. During invasive GAS infection, these mechanisms might contribute to the degradation of extracellular matrix and to the activation of matrix metalloproteases [22, 23]. Concomitant with the production and activation of proteases, GAS must have developed mechanisms to acquire selfprotection against proteolytic degradation. The recruitment of the
human protease inhibitor α2-macroglobulin (α2-M) to the surface of GAS has been proposed as one such potential mechanism [24–26]. Human α2-M is a 725-kDa homotetrameric protein capable of inhibiting a wide range of proteases [27–30]. Interaction between GAS and α2-M seems to be mediated mainly by the streptococcal surface protein G–related α2-macroglobulin–binding (GRAB) protein [26], which was identified by sequence homology to the α2-M–binding domain of protein G from groups C and G streptococci [31–33]. The molecular mass of the core protein is ∼22.8 kDa, and the core protein contains a variable number of 28-aa repeated regions. The α2-M–binding region is localized in the NH2-terminal part of GRAB and is next to a repeat region. The GRAB gene seems to be present in most Streptococcus pyogenes strains and is highly conserved [26], a finding that suggests that the protein GRAB plays a critical role in the physiology of S. pyogenes. A GAS mutant strain devoid of surface-bound GRAB has been shown to be less virulent than the wild-type strain, after intraperitoneal infection of mice, a finding that indicates the potential of the protein GRAB as a virulence determinant of GAS [26]. However, we believed that, because of the ability of GRAB to recruit a protease inhibitor, the expression of GRAB might be advantageous to GAS, in an environment with a high concentration of proteases, such as in soft-tissue infection. Therefore, in this study, we have evaluated the contribution of GRAB to GAS virulence in a mouse skin infection model.

**MATERIAL AND METHODS**

**Bacterial strains and growth conditions.** The blood-isolated S. pyogenes wild-type strain KTL3 (serotype M1) and the derived GRAB-deficient mutant strain MR4 have been described elsewhere [26]. MR4 was generated by the pFW13 suicide vector [34] and is devoid of surface-associated GRAB; instead, it secretes a truncated form that lacks the cell wall–anchoring region [26]. KTL3 was grown in Todd-Hewitt broth (GIBCO) supplemented with 1% yeast extract (Difco), under static conditions at 37°C, or onto blood agar plates (GIBCO). MR4 was grown under similar conditions but was supplemented with 150 μg/mL kanamycin. Both strains exhibit similar growth characteristics in broth. The clinical GAS isolates used for 125I–α2-M–binding studies were obtained from different geographic areas, including Australia, India, and Germany. All strains were isolated after 1993, from patients with skin and throat infections. A high percentage of the strains were M-nontypeable with a highly variable Vir type [35]. The remaining strains exhibited many different M types, a finding that indicates high heterogeneity among the different isolates. Many of the clinical isolates used in this study have been characterized by Goodfellow et al. [36].

**α2-M–binding assay.** The binding of α2-M to S. pyogenes was assessed by use of 125I-labeled protein, according to procedures described elsewhere, and was standardized for groups A, C, and G streptococci [24]. Radiolabeling of α2-M (Sigma) was performed by use of carrier-free 125I (Amersham Pharmacia Biotech), by the chloramine T method, with a specific activity of ∼1 mCi/mg of protein [24, 37]. In brief, bacteria were cultured at 37°C in Todd-Hewitt broth supplemented with 1% yeast extract and were harvested at different points of the growth phase, were washed in PBS containing 0.05% Tween 20, and were incubated with 125I–α2-M for 45 min. Binding assays were conducted in triplicate with ∼1.25 × 10^6 cfu/250 μL and 10 ng of 125I-labeled α2-M. After a washing step and centrifugation, the activity retained in the pellet was measured in a γ-spectrometer and was expressed as percentage of the added activity, as determined by precipitation with trichloroacetic acid. KTL3 and MR4 strains were included as positive and negative controls, respectively.

**ELISA.** After infection with GAS, antibody titers in serum of convalescent patients were determined by ELISA. In brief, 96-well Nunc-ImmunoMaxiSorp assay plates (Nunc) were coated with 50 μL/well His-tag–purified GRAB protein (strain A 82, with grab sequence identical to that of strain SF370), at a concentration of 5 μg/mL, in coating buffer (bicarbonate; pH 8.2). After incubation overnight at 4°C, plates were blocked with 10% fetal calf serum (FCS) in PBS for 1 h at 37°C. Serum diluted 1:50 in 10% FCS-PBS was added (100 μL/well), and plates were incubated for 2 h at 37°C. After 4 washes with PBS containing 0.05% Tween 20, peroxidase-conjugated mouse anti–human IgG (PharMingen) was added, and plates were further incubated, for 2 h at 37°C. After another 4 washes, reactions were developed by ABTS, in 0.1 M citrate-phosphate buffer (pH 4.35) containing 0.01% H2O2. Absorbance was determined at 405 nm in a microtiter reader apparatus.

**Polymerase chain reaction (PCR).** PCR amplification of the grab gene was performed by use of the following oligonucleotides: primer 1, 5’-ATGGGAAAAGAAATAAAAGTGAA-3’ (position 61–87) of the gene, and primer 2, 5’-CTAA-TTTTCTTTGCACTTTGAACCTAC-3’ (position 688–714) of the gene of S. pyogenes reference strain SF370 (ATCC 700294; GenBank accession no., GI:4589078). Chromosomal DNA from the different clinical isolates was used as template, and the manufacturer’s (QIAGEN) instructions were followed.

**Bactericidal assay.** Resistance to phagocytosis was measured in whole human blood by a modification of the Lancefield bactericidal assay for GAS [38]. Mid-log phase bacteria were washed and were serially diluted in PBS. In sterile glass tubes, bacterial suspension (100 μL) containing 10^5 cfu was added to 1 mL of fresh, hiraparinized human blood, and the resultant solution was incubated on an orbital shaker for 1.5 h at 37°C. The survival index was calculated as the colony-forming units.
recovered after the 1.5-h incubation, divided by the initial inoculum added before incubation.

Resistances of both KTL3 and MR4 to phagocytosis were also examined, by use of mouse neutrophils (polymorphonuclear leukocytes [PMNLs]). For this purpose, BALB/c mice were injected with carrageenan (1 mg/mouse) (Sigma) 48 h before PMNL isolation. Carrageenan treatment increases the number of PMNLs and reduces the number of macrophages, in the peritoneal cavity [39]. Peritoneal lavage was then performed by use of 5 mL of Dulbecco’s modified Eagle medium (DMEM)–HEPES (containing 10% heat-inactivated FCS)/mouse, and isolated cells were adjusted to 3 × 10⁶ cells/mL. Mid-log phase bacteria were harvested, were washed, and were diluted in tissue culture medium, to 3 × 10⁶ and 3 × 10⁷ cfu/mL. Resting or phorbol-12-myristate-13-acetate (ICN Biomedicals)–activated PMNLs were combined with equal volumes of bacterial suspension (250 μL:250 μL) and were incubated for 1 h at 37°C on a rotary shaker. Bacteria incubated in medium without PMNLs were used as controls. PMNLs were then lysed with distilled H₂O for 5 min, and serial dilutions were plated on blood agar. The number of viable bacteria was determined and was compared with that of the controls.

**Mouse strains.** Inbred, pathogen-free 8-week-old BALB/c and C3H/HeN mice were purchased from Harlan-Winkelmann.

**Infection model.** Before infection, fur was removed from a 2 × 2-cm area on the backs of mice by use of an electric shaver. Mice were then injected with washed mid-log phase harvested bacteria, at a volume of 100 μL of PBS containing the appropriate inoculum dose. Bacteria were injected by use of a 27-gauge needle, which raised the superficial bleb below the skin. The number of injected microorganisms was determined by spectrophotometry (Novaspec II; Amersham Pharmacia Biotech) and was verified by performance of colony counts on blood agar–plated serial dilutions. The size, after infection, of the skin lesions generated with either KTL3 or MR4 was determined by use of a sliding caliper and a millimeter scale, at intervals of 24 h.

**Determination of bacterial loads in systemic organs.** Mice were infected subcutaneously with 2.5 × 10⁵ cfu of either KTL3 or MR4. Groups of 5 mice were killed at 24, 48, and 72 h after inoculation, and livers and spleens were removed and homogenized in 5 mL of PBS. Organ homogenates were serially diluted in PBS and were plated on blood agar plates. To exclude potential revertants, samples were double-plated on blood agar supplemented with kanamycin. Further confirmation was done by replica plating of the mutant strain obtained from tissue of infected mice.

**Isolation and characterization of phagocytic cells from GAS-infected mice.** BALB/c mice were infected subcutaneously with 5 × 10⁸ cfu of either KTL3 or MR4, and inflammatory cells attracted to the infected site were isolated by subdermal

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**Figure 1.** Capacity of Streptococcus pyogenes KTL3 obtained from different growth phases to bind ¹²⁵I–α₂-macroglobulin (α₂-M). Bacteria were taken from culture at early, mid, and late logarithmic growth phases, as well as at the early and late stationary phases. OD, optical density.

**Figure 2.** Detection of anti–protein G–related α₂-macroglobulin–binding (GRAB) IgG antibodies in serum samples from convalescent group A streptococci (GAS)–infected patients (n = 33) and from uninfected control subjects (n = 8). Serum was obtained from patients with either pharyngitis (P 1–P 3) or skin infections (S 1–S 30) caused by Streptococcus pyogenes. Anti-GRAB IgG was determined by conventional ELISA. Serum obtained from healthy persons was used as negative control (Ctr 1–Ctr 8). OD, optical density.
Figure 3. 

Figure 4. A, Kaplan-Meier survival curves of C3H/HeN mice after subcutaneous challenge with $2.5 \times 10^8$ cfu of Streptococcus pyogenes strain KTL3, protein G–related $\alpha_2$-macroglobulin–binding protein–deficient MR4 mutant strain, or PBS alone (control). Animals were monitored, and deaths were recorded, daily, over 7 days. B and C, Bacterial loads in livers (B) and spleens (C) of C3H/HeN mice at 24, 48, and 72 h after inoculation with $2.5 \times 10^8$ cfu of either KTL3 or MR4. Each time represents a mean of 5 mice/group; bars indicate SEs.

Contribution of GRAB to GAS Virulence

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Figure 3. Ability of Streptococcus pyogenes KTL3 and protein G–related $\alpha_2$-macroglobulin–binding protein–deficient mutant MR4, obtained from cultures at different growth phases, to survive in whole human blood. OD, optical density.

Figure 4. A, Kaplan-Meier survival curves of C3H/HeN mice after subcutaneous challenge with $2.5 \times 10^8$ cfu of Streptococcus pyogenes strain KTL3, protein G–related $\alpha_2$-macroglobulin–binding protein–deficient MR4 mutant strain, or PBS alone (control). Animals were monitored, and deaths were recorded, daily, over 7 days. B and C, Bacterial loads in livers (B) and spleens (C) of C3H/HeN mice at 24, 48, and 72 h after inoculation with $2.5 \times 10^8$ cfu of either KTL3 or MR4. Each time represents a mean of 5 mice/group; bars indicate SEs.

Lavage with 2 mL of HEPES-buffered DMEM containing 10% FCS and 1% each of glutamine, penicillin, and streptomycin and were analyzed by cell cytometry, to determine the percentage of macrophages to neutrophils. Cell cytometry was performed by use of phycoerythrin-conjugated anti-mouse RB6 and fluorescein isothiocyanate–conjugated anti-mouse F480 antibodies (PharMingen).

Tissue collection and histology. Mice were injected subcutaneously with $5 \times 10^8$ cfu of either KTL3 or MR4, and skin lesions were removed after 48 h of inoculation, by wide marginal excision around the injection site. Tissue sections were fixed in 10% phosphate-buffered formaldehyde for 24 h, were washed extensively, and were stored in 70% ethanol. Tissue dehydration and paraffin embedding were performed according to standard protocols [40]. Samples were then sectioned to 7-µm slices by a rotary microtome (Leica RM 2135), were stained with buffered azure–eosin, and were examined by an Axioskop microscope (Zeiss).

For immunofluorescence staining, fixed and immobilized sections were rehydrated according to standard protocols [40] and were placed in distilled H₂O. After blocking for 1 h with PBS containing 10% heat-inactivated FCS, samples were overlaid with rabbit polyclonal anti–S. pyogenes serum [41] (1:100), in PBS with 1% FCS, for 1 h at room temperature. Unbound antibodies were removed by immersion of the samples in PBS. In a second step, sections were incubated with a tetramethyl rhodamine isothiocyanate–conjugated polyclonal goat anti–rabbit IgG (Sigma; 1:200), in PBS with 1% FCS, for 1 h, were extensively washed with PBS, and were mounted. Immunofluorescence was then visualized by a fluorescence microscope (Axioskop; Zeiss).

Electron microscopy samples were fixed in a fixation solution of 0.2% glutaraldehyde and 0.5% formaldehyde, in cacodylate buffer (pH 6.9; 0.1 M cacodylate, 0.09 M sucrose, 0.01 M MgCl₂, and 0.01 M CaCl₂), for 1 h on ice. After several washing steps with cacodylate buffer containing 10 mM glycine, samples were...
dehydrated according to the progressive-lowering-of-temperature method, by use of a graded series of ethanol: 10% ethanol on ice, 30% ethanol at −20°C, and 50%–100% ethanol at −30°C. Samples were then infiltrated with Lowicryl K4M resin (1 part ethanol:1 part K4M overnight, 1 part ethanol:2 parts K4M for 24 h, and pure K4M resin for 48 h, with several changes). Samples were polymerized by UV light (366 nm) for 2 days at −30°C and were further polymerized by UV light for another 2 days, at room temperature. Samples were cut with a diamond knife and were collected onto polyvinyl formal–coated 300-mesh copper grids (Fluka). Grids were then incubated with a 1:25 dilution of the anti-GRAB antibody (stock solution, 1.8 mg/mL of IgG protein) for 12 h at 4°C. They were then washed with PBS, were incubated with protein A–gold complexes (15 nm in diameter) for 30 min at room temperature (BritishBiocell), were washed again, with PBS containing 0.1% Tween 20, were subsequently washed in distilled water, and were air-dried. Counterstaining was performed, by use of 4% aqueous uranyl acetate, for 5 min. Samples were then examined in an Oberkochen transmission electron microscope (EM910; Zeiss) at an acceleration voltage of 80 kV.

Statistical analysis. Statistical significance between samples was determined by Student’s t test.

RESULTS

\( \alpha_2 \)-M binding among skin and throat GAS isolates. Eighty-six strains of \textit{S. pyogenes} isolated from patients with either throat infection (40) or skin infection (46) were tested for their capacities to bind \(^{125}\text{I}\)–labeled \( \alpha_2 \)-M. Strains binding \( >10\% \) of the original added activity were considered to be displaying binding activity. Twenty isolates (23%) bound \( \alpha_2 \)-M, whereas 66 isolates (77%) did not. Studies of the correlations between the source of the isolates and their capacities to bind \( \alpha_2 \)-M showed that 80% of strains binding \( \alpha_2 \)-M were skin isolates, a finding that suggests a preferential expression of protein GRAB during skin infection. However, it is possible that the strains not binding \( \alpha_2 \)-M had lost this function after subculture under laboratory conditions. Thus, the clinical GAS isolates were genotyped for the presence of the \textit{grab} gene by PCR amplification with \( \textit{grab} \)-specific oligonucleotide primers. All of the strains tested contained the gene, a finding that indicates the relevance of the protein GRAB for the biology of the bacterium.

\( \alpha_2 \)-M–binding activities during the different bacterial growth phases. The binding capacity of KTL3 was determined during various growth phases by kinetic studies with \(^{125}\text{I}\)-labeled \( \alpha_2 \)-M. MR4 was used as negative control, to rule out nonspecific binding to other bacterial determinants. Max-
Figure 6.  
A, Flow cytometric analysis of inflammatory neutrophils (PMNLs) (RB6) and macrophages (F480) present in skin lesions of BALB/c mice infected with either *Streptococcus pyogenes* KTL3 (upper panels) or protein G–related α2-macroglobulin–binding (GRAB) protein–deficient mutant MR4 (lower panels) at 48 h of infection (solid line). Isotype-matched antibodies served as controls in all experiments (dotted line). B, C, and D, Histopathologic analyses of dermal sections taken from BALB/c mice at 48 h after inoculation with either KTL3 (B) or MR4 (C). Sections were stained with eosin–azure blue. Bacteria are indicated with arrows. Insets in B and C show higher magnification of inflammatory PMNLs. Skin sections were also stained with tetramethyl rhodamine isothiocyanate–conjugated GAS-specific antibodies and were visualized by immunofluorescence microscopy (D). Lower-magnification (left) and higher-magnification (right) photographs are shown. Five mice per group were used for histopathologic studies.
imal $\alpha_2$-M-binding activity of KTL3 was observed during the early exponential growth phase, and activity slowly declined until reaching a plateau (40% binding activity) during the stationary phase (figure 1).

**Detection of anti-GRAB antibodies in serum of convalescent patients.** A few weeks after diagnosis, serum samples were collected from convalescent patients with either pharyngitis or skin infection caused by *S. pyogenes*. Serum samples were assayed for the presence of anti-GRAB IgG antibodies. As shown in figure 2, significantly higher levels of anti-GRAB antibodies were found in the serum samples of all patients tested than in the serum samples of healthy subjects from the control group, which included persons from the same geographic area who were seronegative for GAS antibodies by whole-cell ELISA.

**Resistance to phagocytosis of MR4.** The possibility that protein GRAB can contribute to the antiphagocytic capacity exhibited by GAS was then examined. For this purpose, survival of both KTL3 and MR4 were measured in fresh human blood from 3 separate donors, by a modified Lancefield bactericidal assay. Both strains were equally resistant to phagocytic killing in whole blood. No differences in antiphagocytic activity were found between MR4 and KTL3, both of which had been obtained from cultures at different growth phases (figure 3). Similar results were obtained in killing assays by use of resting or phorbol-12-myristate-13-acetate–activated mouse PMNLs (data not shown).

**Survival times of mice after subcutaneous infection with KTL3 or the MR4 strains of *S. pyogenes*.** Groups of 10 C3H/HeN mice were infected subcutaneously with $2.5 \times 10^8$ cfu of
either KTL3 or MR4, and survival was monitored over a 7-day period. As shown in figure 4A, at day 7 after inoculation, 90% of mice infected with MR4 survived infection, whereas only 40% of mice infected with KTL3 were still alive.

To determine whether increased survival was associated with a lower rate of systemic bacterial growth, bacterial burden in liver and spleen was determined at 24, 48, and 72 h after inoculation. Results in figure 4B and 4C show that bacterial dissemination from skin to systemic organs was comparable in both MR4 and KTL3, at 24 h after infection. From this time on, infection was stabilized in mice infected with MR4, and bacterial clearance could be observed to some extent in the spleens of these mice. In contrast, progressive bacterial growth was observed in systemic organs of mice infected with KTL3. All bacteria recovered, at different times after infection, from the organs of mice infected with MR4 remained resistant to kanamycin, as determined by double-plating in both the presence and the absence of the antibiotic and also by replica plating.

*S. pyogenes* KTL3 *generates larger lesions than does MR4, after subcutaneous infection of mice*. In a first set of experiments, C3H/HeN mice were infected subcutaneously with either KTL3 or MR4, and the development of skin lesions was monitored on a daily basis. A white intensely inflamed area was observed as soon as 24 h after inoculation. As the infection progressed, the local inflammation expanded faster in mice infected with KTL3 than it did in those infected with MR4, giving rise to much larger lesions in mice in the former group (figure 5A). These differences were also evident when half of the inoculum dose was used (figure 5A). To rule out an influence of mouse background on the differences observed between KTL3 and MR4, BALB/c mice, which are very resistant to GAS infection [42], were infected subcutaneously with $5 \times 10^8$ cfu of either KTL3 or MR4. Similar to what was observed in C3H/HeN mice, in BALB/c mice, infection with MR4 generated smaller and more superficial lesions than did infection with KTL3 (figures 5B and 5C).

**Histopathologic examination of skin lesions.** BALB/c mice were infected subcutaneously with $5 \times 10^9$ cfu of either KTL3 or MR4, and skin sections were taken at 48 h after inoculation, for histopathologic examination. Stained sections of skin isolated from both groups of mice revealed a strong inflammatory response, mainly composed of an intense infiltration of PMNLs, a finding that was confirmed by cell cytometric analysis (figure 6A). High densities of streptococci can be observed at the inoculation sites of mice infected with either KTL3 (figure 6B) or MR4 (figure 6C). However, in tissue sections from MR4-infected mice, the infection seems to be contained at the infection site by a thick surrounding layer of inflammatory cells (figure 6C). In contrast, in KTL3-infected mice, the infection was widely spread in epidermis, dermis, and subcutis (figure 6B). Invasion of the deepest layers of the skin was also more pronounced in skin infected with the KTL3 strain (figure 6B).

The presence of high numbers of *S. pyogenes* in skin lesions was further demonstrated by immunofluorescence staining with GAS-specific serum antibodies (figure 6D). Additional presence of protein GRAB in the surface of KTL3 was confirmed by electron microscopy (figure 7A). The surface MR4 was included as control (figure 7B).

**DISCUSSION**

The results presented here indicate that GRAB plays an important role in streptococcal virulence, because, in a mouse model of skin infection, the presence of surface-associated protein GRAB confers a survival advantage to *S. pyogenes*. That the *grab* gene is present in all clinical isolates tested [26] (this study) and that anti-GRAB antibodies are present in the serum of convalescent GAS-infected patients provide further evidence for a role of the protein GRAB during the infection process.

*S. pyogenes* is generally an extracellular pathogen that colonizes either the mucosal epithelium of the upper respiratory tract or the epidermis of the skin. Local infections caused by *S. pyogenes*, as well as particular skin infections, are characterized by both an extensive infiltration of PMNLs and serum extravasations [43]. Although recruited PMNLs are important for bacterial clearance [44], neutrophils, by the release of oxygen-free radicals and proteases, also contribute to tissue damage [45]. In addition, during soft-tissue infection, GAS not only produce proteases and other products for degradation of the extracellular matrix [8, 12, 14, 46], but they also activate host metalloproteases [23]. Therefore, in these ecological niches, survival and persistence of *S. pyogenes* is ensured by a number of strategies directed to circumvent the host immune system (e.g., antiphagocytic mechanisms) and probably also to acquire protection from a harsh environment [38, 47]. Regarding this last point, the recruitment of the protease inhibitor $\alpha_2$-M to the surface of GAS has been proposed as a potential mechanism for bacterial protection against proteolytic degradation [26, 48]. $\alpha_2$-M is present in high concentrations in serum [29] and can be transported to the site of GAS infection, after serum extravasation, during the inflammatory reaction. That binding of $\alpha_2$-M is predominant among GAS strains isolated from skin infections underlines the importance of $\alpha_2$-M binding activity of GAS during skin infection. Binding of $\alpha_2$-M to GAS is mainly mediated by the protein GRAB [26]. In the present study, we have shown that, in a mouse model of skin infection, a GRAB-deficient mutant strain of *S. pyogenes* (MR4) is attenuated in virulence, compared with the wild-type strain (KTL3). This finding supports a previous report showing partial attenuation in virulence of MR4 after intraperitoneal infection of mice [26]. The mechanism by which recruitment of $\alpha_2$-M confers survival...
advantage to \textit{S. pyogenes} is not yet clear. However, it can be hypothesized that binding of \(\alpha_c\)-M to the bacterial surface might contribute, on the one hand, to removal of \(\alpha_c\)-M from the environment and, thus, to maintenance of the effective activity of the proteases for more efficient tissue spreading and, on the other hand, to protection of the bacterial surface proteins from the action of these proteases. Support for these notions is provided by the finding that GAS is unable to bind \(\alpha_c\)-M when \(\alpha_c\)-M is already coupled with the proteases [24, 25]. However, because MR4 is deficient only in surface-bound GRAB but is still able to release GRAB in the infection milieu [26], the survival advantage of KTL3 that has been observed in the mouse skin infection might be mainly due to the protection from protease degradation conferred by the presence of GRAB on the bacterial surface. Thus, exposure of M protein and other surface proteins involved in antiphagocytic activity to the activities of proteases might facilitate the uptake of MR4 by the PMNLs present at the site of infection. Further support for this view is provided by the preferential expression of GRAB during logarithmic growth, expression that matches the expression of the antiphagocytic M protein and C5a peptidase [8].

In conclusion, our results indicate that protein GRAB, the only \(\alpha_c\)-M–binding protein identified to date for \textit{S. pyogenes}, is important during the pathogenesis of skin infection. These results highlight the potential of the protein GRAB as a vaccine candidate against GAS infections.

References


