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A natural variant of the cysteine protease virulence factor of group A *Streptococcus* with an arginine-glycine-aspartic acid (RGD) motif preferentially binds human integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$

KATHRYN E. STOCKBAUER*†, LORANNE MAGOUN‡, MENGYAO LIU*, EUGENE H. BURNS, JR.*§, SIDDESWAR GUBBA*, SARAH RENISH*¶, XI PAN*, SARAH C. BODARY‖, ELIZABETH BAKER**, JENIFER COBURN††, JOHN M. LEONG‡, AND JAMES M. MUSSER*‡‡

*Institute for the Study of Human Bacterial Pathogenesis, Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; ‡Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01655; ‖Department of Immunology, Genentech, Inc., South San Francisco, CA 94080; **Department of Vascular Biology, Scripps Research Institute, La Jolla, CA 92037; and ††Division of Rheumatology and Immunology, Department of Medicine, Tufts-New England Medical Center, Boston, MA 02111

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ABSTRACT **The human pathogenic bacterium group A *Streptococcus* produces an extracellular cysteine protease [streptococcal pyrogenic exotoxin B (SpeB)] that is a critical virulence factor for invasive disease episodes. Sequence analysis of the *speB* gene from 200 group A *Streptococcus* isolates collected worldwide identified three main mature SpeB (mSpeB) variants. One of these variants (mSpeB2) contains an Arg-Gly-Asp (RGD) sequence, a tripeptide motif that is commonly recognized by integrin receptors. mSpeB2 is made by all isolates of the unusually virulent serotype M1 and several other geographically widespread clones that frequently cause invasive infections. Only the mSpeB2 variant bound to transfected cells expressing integrin $\alpha_v\beta_3$ (also known as the vitronectin receptor) or $\alpha_{IIb}\beta_3$ (platelet glycoprotein IIb-IIIa), and binding was blocked by a mAb that recognizes the streptococcal protease RGD motif region. In addition, mSpeB2 bound purified platelet integrin $\alpha_{IIb}\beta_3$. Defined β_3 mutants that are altered for fibrinogen binding were defective for SpeB binding. Synthetic peptides with the mSpeB2 RGD motif, but not the RSD sequence present in other mSpeB variants, blocked binding of mSpeB2 to transfected cells expressing $\alpha_v\beta_3$ and caused detachment of cultured human umbilical vein endothelial cells. The results (*i*) identify a Gram-positive virulence factor that directly binds integrins, (*ii*) identify naturally occurring variants of a documented Gram-positive virulence factor with biomedically relevant differences in their interactions with host cells, and (*iii*) add to the theme that subtle natural variation in microbial virulence factor structure alters the character of host-pathogen interactions.**

Group A *Streptococcus* (GAS) is a human pathogenic bacterium that causes diverse infections ranging in severity from relatively mild pharyngitis to life-threatening toxic shock syndrome and necrotizing fasciitis (1). GAS is composed of a heterogeneous array of chromosomal genotypes, and substantial levels of allelic variation also exist in genes encoding putative and proven virulence factors that mediate host-pathogen interactions (2–5). With the exception of M protein, whose structural variation helps GAS evade the type-specific immune response of the host (6), there has been little investigation of the potential ramifications of GAS allelic variation for host-pathogen interactions.

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GAS isolates produce a highly conserved extracellular cysteine protease known as streptococcal pyrogenic exotoxin B (SpeB) (reviewed in ref. 5). SpeB is initially expressed as a 40-kDa zymogen but then is converted to a 28-kDa active protease by proteolytic truncation (5, 7). SpeB is a critical virulence factor in two mouse models of invasive disease (8, 38) and human infections (5). Purified SpeB causes a cytopathic effect on cultured human endothelial cells (3) and has been shown to activate a host matrix metalloprotease (9).

Integrins are heterodimeric membrane proteins located on the surface of mammalian cells that participate in cell–cell adhesion, and cellular differentiation, migration, and attachment to the extracellular matrix (10). The 3-aa sequence Arg-Gly-Asp (RGD) is critical for ligand recognition by many integrins (11). Microbial pathogens as diverse as *Borrelia burgdorferi*, *Yersinia* spp., *Bordetella pertussis*, adenovirus, foot-and-mouth disease virus, and hantaviruses bind integrins, usually through an RGD motif located on cell-surface proteins (12–16). Several of these proteins are proven virulence factors (14–16).

Here, we report the identification of a naturally occurring mature SpeB (mSpeB) variant that binds host cell integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ through an RGD motif. The RGD-containing variant is made by M1 isolates, the most common GAS serotype recovered from human invasive infections worldwide (17), and by several other geographically widespread clones that frequently cause invasive infections.

MATERIALS AND METHODS

Bacterial Isolates and DNA Sequence Analysis of *speB*. GAS isolates ($n = 200$) cultured from patients in 41 countries were studied. Seventy-seven M protein serotypes or provisional M serotypes were represented. Thirty-eight of 106 isolates that were serologically nontypeable with M protein antisera had a unique *emm* sequence. The *speB* DNA sequence data were generated, assembled, and edited as described (3).

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Abbreviations: GAS, group A *Streptococcus*; SpeB, streptococcal pyrogenic exotoxin B; mSpeB, mature SpeB; zSpeB, SpeB zymogen; CHO, Chinese hamster ovary; HUVEC, human umbilical vein endothelial cell.

†Present address: Department of Microbiology and Immunology, University of California Los Angeles School of Medicine, Los Angeles, CA 90024.
§Present address: Department of Biology, Drew University, Madison, NJ 07940.
¶Present address: Department of Anthropology, Pennsylvania State University, University Park, PA 16802.
‡‡To whom reprint requests should be addressed. e-mail: jmusser@bcm.tmc.edu.

Purification of Streptococcal Cysteine Protease Variants and Recombinant Zymogen. Cysteine protease variants were purified from culture supernatants (18). Strains MGAS 4160 (serotype M77), MGAS 1719 (serotype T8), and MGAS 4794 (serologically nontypeable) were used as the sources for mSpeB1, mSpeB2, and mSpeB3, respectively. The purified proteins were >95% pure, as analyzed by SDS/PAGE and staining with Coomassie brilliant blue. Recombinant C192S mutant zymogen (designated zSpeB2^{C192S}) was purified from *Escherichia coli* (7). The C192S amino acid substitution replaces the active site cysteine residue and results in a molecule that is proteolytically inactive (7). A 28-kDa mature form of this proteolytically inactive mutant (designated mSpeB2^{C192S}) was made from zSpeB2^{C192S} by trypsin digestion (7). zSpeB2^{C192S} was electrophoretically pure as assessed by SDS/PAGE and silver staining.

Synthetic Peptides and Antibodies. Synthetic peptides corresponding to amino acid residues 305–311 in SpeB (INRGDFS and INRSDFS) and negative control peptides (INRGEFS and GIESFNR) were purchased from Chiron. Each peptide was synthesized with its carboxyl terminus in the amide form and purified by HPLC. Mouse mAb 2A3-B2-C12 recognizes the sequence 302-VHQINR-307 in protease variants SpeB1 and SpeB3 and the sequence 305-INRG-308 in variant SpeB2 (19). Rabbit antiserum to SpeB was made by Bethyl Laboratories (Montgomery, TX). Anti-Mac1 mAb 44a was provided by L. Zhang (American Red Cross). Anti-$\alpha_v\beta_3$ mAb LM609, anti-$\alpha_{IIb}\beta_3$ (clone P2) mAb, and rabbit polyclonal anti-$\alpha_v\beta_3$ antibody were purchased from Chemicon and GIBCO/BRL, respectively.

Enzyme Kinetic Analysis. Enzyme kinetic analysis was performed with purified 28-kDa protease wild-type variants with azocasein (Sigma) as substrate. Before kinetic analysis, the azocasein was dissolved in PBS at a concentration range of 0.1 mg/ml to 1.4 mg/ml. K_m, V_{max}, and K_m/V_{max} were calculated by standard procedures.

Mammalian Cell Culture. Chinese hamster ovary (CHO)-$\alpha_{IIb}\alpha_{L\Delta}$ β_3 cells are transfected CHO-K1 cells that express the wild-type β_3-chain and an $\alpha_{IIb}/\alpha_{L\Delta}$ chimera consisting of the extracellular and transmembrane domains of α_{IIb} fused to an internal-deletion derivative of the cytoplasmic domain of the α_L subunit. The resulting receptor is in a constitutively active conformation (20). CHO-β_3-A252V, CHO-β_3-D119Y, CHO-β_3-R740X, and CHO-β_3-E312K express extracellular and transmembrane domains of α_{IIb} and β_3 with defined amino acid changes (21). CHO-K1 cells, transfected CHO cells, human kidney 293 cells, and an $\alpha_v\beta_3$-transfected derivative of the 293 cell line (cell line 835) were cultured as described (20, 22, 23).

ELISA assays were used to compare the level of expression of $\alpha_{IIb}\beta_3$ by CHO cells transfected with genes encoding altered β_3 chains. Cells growing in microtiter wells were washed once with RPMI medium 1640/1% BSA and incubated with 50 μl of 0.6 μg/ml anti-α_{IIb} mAb (Immunotech) per well for 45 min at room temperature. The wells were washed twice with RPMI medium 1640/1% BSA and then 50 μl of horseradish peroxidase-conjugated anti-mouse IgG (1:10,000 dilution; Promega) for 45 min at room temperature. The wells were washed three times with RPMI medium 1640/1% BSA, and 100 μl of TMB peroxidase substrate (Kirkegaard & Perry Laboratories) was added to each well. The ELISA signal was read at 620 nm. In parallel, the number of cells per well was quantitated by crystal violet staining (24), and the ELISA signal was normalized to the cell number. CHO cells transfected with genes encoding the mutant altered β_3 chains expressed $\alpha_{IIb}\beta_3$ between 62% and >90% of the level of receptor on CHO-$\alpha_{IIb}\alpha_{L\Delta}$ β_3 cells.

Attachment of Mammalian Cells to Purified SpeB Proteins. Attachment of mammalian cells to integrin ligands was assayed as described (24, 25). Briefly, purified SpeB proteins or *Yersinia pseudotuberculosis* invasin protein were immobilized overnight at 4°C in 96-well microtiter dishes (Flow Laboratories) at a concentration of 1.25–2.0 μg/ml in 50 mM carbonate-bicarbonate buffer, pH 9.6, and then blocked for 2 h at 20°C in PBS supplemented with 0.2% BSA. Dispersed mammalian cells (1×10^5) in 100 μl of RPMI medium 1640 supplemented with 20 mM Hepes, pH 7.0 and 0.4% BSA were added to each well and allowed to bind for 1 h at 37°C. Stably bound cells were quantitated by crystal violet staining (24). To examine the effect of anti-integrin antibodies, cells were incubated with 10 μg/ml of antibody for 1 h at 20°C before binding to immobilized substrates. To examine the effect of anti-SpeB antibodies, mouse ascites containing mAb 2A3-B2-C12 or control mouse ascites were diluted in RPMI medium 1640 supplemented with 20 mM Hepes, pH 7.0, and 0.4% BSA, and incubated in SpeB2-coated microtiter wells for 1 h at 20°C.

Cultures of Human Umbilical Vein Endothelial Cells (HUVECs) and Treatment with Synthetic Peptides. HUVECs were isolated from fresh cords, grown to confluency in complete M199 medium with 20% fetal calf serum (HyClone), and used in passages 3–5 (9). The HUVECs were washed twice with Hank's balanced salt solution (GIBCO/BRL), and 2 ml of complete serum-free medium containing each synthetic peptide (20 μg/ml) or active streptococcal cysteine protease (10 μg/ml) were added for 24 h. Detached cells were removed by washing.

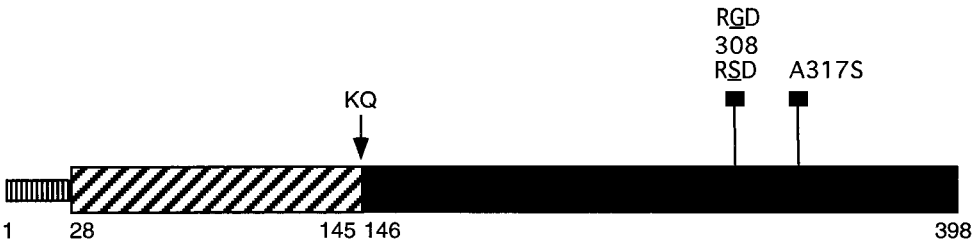
Binding of Purified Integrin by SpeB Proteins. Integrin $\alpha_{IIb}\beta_3$ was purified from human platelets by RGD-Sepharose chromatography (23). SpeB binding to the purified integrin was measured by ELISA as described (24, 26). Briefly, Linbro ELISA plates (96-well; Flow Laboratories) were coated with integrin ligands overnight at 4°C with 2.5 μg/ml of mSpeB2 and blocked for 2 h at 20°C with PBS supplemented with 3.5% BSA. Purified $\alpha_{IIb}\beta_3$ integrin was added at a concentration of 2.5 μg/ml in 25 mM Hepes, pH 7.0, 125 mM NaCl, 1 mM $MnCl_2$, and 20 mM octyl glucoside. The plate was incubated for 3 h at 20°C, washed, and fixed with 3% paraformaldehyde, and the bound integrin was revealed by an ELISA with rabbit antivitronectin receptor (24).

RESULTS

Allelic Variation in *speB*. Eighty-nine *speB* alleles were identified among the 200 GAS isolates. There were 55 polymorphic nucleotide sites in the coding region of the gene, including 18 that would result in amino acid replacements. Two of these 18 nonsynonymous substitutions were in the leader peptide and would not be present in the secreted SpeB zymogen.

Proteolytic cleavage between Lys-145 and Gln-146 of the 40-kDa zymogen releases a 12-kDa propeptide and forms the 28-kDa mature cysteine protease (5, 7). It therefore was reasonable to treat the products of the *speB* gene as three distinct molecules for purposes of data analysis and interpretation. Thus, the 89 *speB* alleles would encode 21 different 40-kDa SpeB zymogens, but only three mature forms of SpeB (arbitrarily designated mSpeB1, mSpeB2, and mSpeB3) would be made by 93% of all isolates studied (Fig. 1). The sequences of the three common mSpeB variants differed only by amino acid residues present at position 308 or 317: mSpeB1 (S308, A317), mSpeB2 (G308, A317), and mSpeB3 (S308, S317).

Inspection of the inferred amino acid sequences of the SpeB variants identified a S308G amino acid replacement that transformed an RSD sequence present in mSpeB1 and mSpeB3 into an RGD motif in mSpeB2. The mSpeB2 variant is made by GAS isolates expressing serotype M1, T8, M13, M33, M44, M46, M60, M64, M68, M72, M74, M76, PT2195, PT4245, and TR2612. Many of these organisms are highly divergent from one another in overall chromosomal character (3, 4), indicating that they did not share a recent ancestor.



Mature Form	Variant Amino Acids	*n*	%
mSpeB1	Ser308, Ala317	118	59
mSpeB2	Gly308, Ala317	40	20
mSpeB3	Ser308, Ser317	28	14

FIG. 1. Schematic summarizing SpeB variation identified in 200 GAS isolates collected worldwide. The leader sequence (amino acids 1–27), propeptide (28–145), and mature protease (146–398) are shown. Proteolytic cleavage between K145 and Q146 of the 40-kDa zymogen releases a 12-kDa propeptide and creates the 28-kDa mature cysteine protease. Three main mature forms (mSpeB1, mSpeB2, and mSpeB3) differ only by amino acid residues present at position 308 or 317: mSpeB1 (S308, A317), mSpeB2 (G308, A317), and mSpeB3 (S308, S317). *n*, number of isolates with designated mature variant; %, percent of all isolates studied with the designated mature variant.

Enzyme Kinetics. The three common mSpeB variants were examined for differences in enzyme kinetic parameters. With azocasein as substrate, we determined that the K_m and V_{max} values for mSpeB1, mSpeB2, and mSpeB3 were identical (data not shown).

Binding of SpeB2 (RGD) Proteins to Integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ Expressed on the Surface of Transfected Cells. Inasmuch as many integrins recognize ligands containing RGD motifs, the hypothesis that mSpeB2 binds members of this receptor family was examined. Integrin $\alpha_v\beta_3$ is expressed on the surface of many human cell types, including endothelial cells (27). The related integrin $\alpha_{IIb}\beta_3$ is expressed exclusively on megakaryocytes and platelets and is critical for platelet aggregation at sites of endothelial damage (28). Both integrins bind ligands containing an RGD motif (29).

SpeB2 protein binding to $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ expressed on the surface of human cells was examined with transfected cell lines. The RGD-containing SpeB2 proteins (mSpeB2, mSpeB2^{C192S}, and zSpeB2^{C192S}) bound to 835 cells, a transfected cell line expressing integrin $\alpha_v\beta_3$ (22), but not to the parental cell line (Fig. 2*A*). Invasin (which binds several integrins with β_1-chains; ref. 30) bound both cell lines. Binding of mSpeB2 to transfected cells was inhibited by a mAb directed against $\alpha_v\beta_3$ but not by a control mAb directed against integrin $\alpha_{Mac}\beta_2$ (Fig. 2*B*). Similarly, the RGD-containing SpeB2 proteins bound to CHO-$\alpha_{IIb}\alpha_{L\Delta}\beta_3$ cells (which express the extracellular domain of $\alpha_{IIb}\beta_3$ in the active conformation), but not to untransfected parental CHO-K1 cells (Fig. 2*C*). Binding of these RGD-containing proteins was inhibited by a mAb directed against $\alpha_{IIb}\beta_3$ but not by a control antibody (Fig. 2*D*).

Hence, two lines of evidence indicated that the SpeB2 RGD motif is critical for binding of β_3-chain integrins on the host cell surface. First, SpeB1, which differs from SpeB2 by only one amino acid (S308G), bound poorly to 835 and CHO-$\alpha_{IIb}\beta_3$ cells (Fig. 2*A*). Second, a mAb that recognizes residues 302–308, a sequence that includes part of the RGD motif in SpeB2, blocked binding of SpeB2 to both 835 and CHO-$\alpha_{IIb}\alpha_{L\Delta}$ β_3 cells (Fig. 2 *B* and *D*).

Decreased Host Ligand Binding and Decreased SpeB Binding Caused by Integrin Mutations. If SpeB2 and host ligands such as fibrinogen recognize integrins similarly, then integrin mutations that diminish host ligand binding (21) also should detrimentally alter SpeB2 binding. Hence, the ability of SpeB2 to bind CHO cells expressing β_3-chain mutants was examined. Two cell lines that express mutant β_3-chains that alter the presumed host-ligand binding site (β_3-A252V and β_3-D119Y) bound to zSpeB2^{C192S} and mSpeB2 more than 20-fold less efficiently than did CHO cells expressing fully functional $\alpha_{IIb}\beta_3$ (Table 1). Mutants that block integrin activation (β_3-R740X and β_3-E312K) also had a substantially reduced ability to bind SpeB2 proteins.

RGD-Containing SpeB Peptides Cause HUVEC Detachment and Inhibit Host Cell and Purified Integrin Binding to mSpeB2. The hypothesis that the RSD ↔ RGD polymorphism conferred other functional differences was examined in two additional assay systems. A synthetic heptapeptide corresponding to residues 305–311 of SpeB2 (INRGDFS) was tested for its ability to inhibit host cell binding to SpeB2. As shown in Fig. 3, this peptide blocked binding of $\alpha_v\beta_3$-positive 835 cells to SpeB2, whereas the corresponding SpeB1 peptide (INRSDFS) had no effect on binding. These synthetic peptides then were assessed for their ability to interfere with HUVEC attachment *in vitro*. The rationale for this experiment was based on the observation that engagement of integrin receptors induces cell rounding and detachment from culture surfaces (31, 32). Peptide INRGDFS caused HUVEC detachment, whereas the INRSDFS peptide and a control peptide (GIESFNR) did not. Removal of the INRGDFS peptide resulted in cell reattachment (data not shown). Taken together, these data show that the detachment effect of the INRGDFS peptide was specific and not the result of toxicity.

To demonstrate that the RGD-containing SpeB peptide inhibited 835 cell attachment by blocking a direct interaction between integrin and SpeB, we tested the ability of the peptide to inhibit binding of purified $\alpha_{IIb}\beta_3$ to SpeB2. $\alpha_{IIb}\beta_3$ bound to microtiter wells coated with mSpeB2 but not to uncoated wells (Fig. 4). As expected, this specific binding was diminished significantly better ($P < 0.005$) by the RGD-containing SpeB2 peptide (INRGDFS) than by a control peptide (INRGEFS) that differs by only one conservative amino acid replacement in the RGD motif (Fig. 4).

DISCUSSION

Integrin Binding and Pathogen Fitness. In this study we identified a Gram-positive virulence factor that directly binds human integrins and discovered that a naturally occurring single-amino acid replacement in a critical GAS virulence

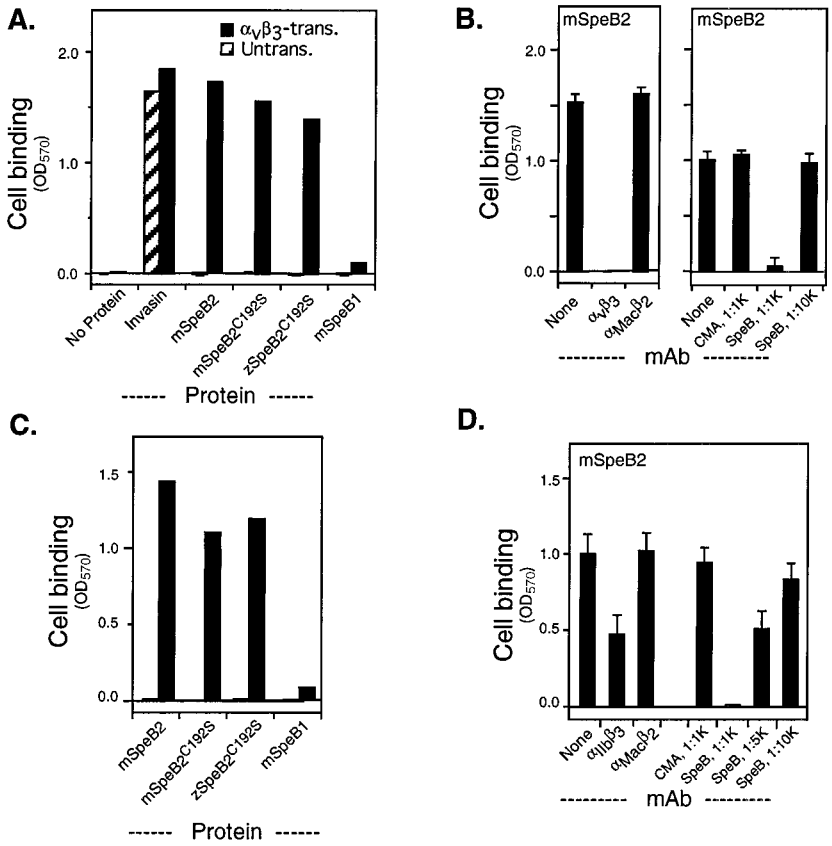


FIG. 2. SpeB2 (RGD containing) binds to integrin $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ expressed on mammalian cells. (*A*) Binding by untransfected cells (hatched bars) or transfected cells expressing $\alpha_v\beta_3$ (solid bars) to microtiter wells coated with buffer alone or with the indicated protein was determined (see *Materials and Methods*). Data shown are the means and SD of quadruplicate samples. (*B*) (*Left*) $\alpha_v\beta_3$-transfected cells were incubated with anti-$\alpha_v\beta_3$ mAb, anti-$\alpha_{Mac}\beta_2$ mAb, or buffer alone before binding to mSpeB2. (*Right*) Control mouse ascites or ascites containing mAb 2A3-B2-C12 directed against amino acid residues 302–308 of SpeB were added before cell binding to mSpeB2-coated wells at the indicated concentrations. The means and SD of quadruplicate samples are shown. (*C*) Binding by untransfected cells (hatched bars) or transfected cells expressing functional $\alpha_{IIb}\beta_3$ (solid bars) to microtiter wells coated with the indicated protein was determined. Data are expressed as the means of duplicate samples. (*D*) Transfected cells expressing functional $\alpha_{IIb}\beta_3$ were incubated with anti-$\alpha_{IIb}\beta_3$ mAb, anti-$\alpha_{Mac}\beta_2$ mAb, or buffer alone before binding to mSpeB2. Alternatively, control mouse ascites or ascites containing mAb 2A3-B2-C12 directed against amino acid residues 302–308 were added to mSpeB2-coated wells at the indicated concentrations before cell binding. Shown are the means and SD of quadruplicate samples. CMA, control mouse ascites; 1:1K, 1:1,000 dilution; 1:10K, 1:10,000 dilution.

factor mediates biomedically relevant differences in host cell interactions. Many GAS isolates with the RGD motif in SpeB are highly divergent in overall chromosomal character (3, 4), which means they have not shared a recent ancestor. SpeB is chromosomally encoded, rather than carried on a mobile element. Therefore, the most likely explanation for the presence of the RGD motif in GAS isolates that are otherwise distantly related is mutational convergence, an occurrence that is strong evidence that the RGD motif enhances long-term fitness and durability. Two important facts provide evidence that mutational convergence to integrin binding is a common theme in the survival strategies used by pathogenic microbes. First, proteins that bind host integrins and have RGD motifs are expressed by pathogens as diverse as Gram-negative and Gram-positive bacteria, viruses, and eukaryotic parasites (10, 12–16). Second, several microbial proteins that bind integrins lack an RGD motif (12, 13, 24, 32), which means that the survival premium to the organism is conferred simply by the phenotype of integrin binding.

Table 1. Integrin mutations that alter function decrease SpeB binding

$\alpha_{IIb}\beta_3$ expressed†	Defect in function‡	Relative binding*	
		zSpeB2	mSpeB2
Wild type	None	1.00	1.00
None	NA	0.00	0.00
β3-A252V	Ligand binding	0.05	0.04
β3-D119Y	Ligand binding	0.00	0.00
β3-R740X	Integrin activation	0.00	0.00
β3-E312K	Integrin activation	0.16	0.23

*Duplicate microtiter wells were coated with SpeB proteins at a concentration (0.25 μg/ml) that gave approximately half-maximal cell binding of CHO-$\alpha_{IIb}\alpha_{L\Delta}\beta_3$, a transfected cell line that expresses functional $\alpha_{IIb}\beta_3$. Relative binding was defined as the ratio of average binding by CHO cells expressing mutant $\alpha_{IIb}\beta_3$ to average binding by CHO cells expressing functional $\alpha_{IIb}\beta_3$. This experiment was performed twice with similar results.

†Untransfected CHO-K1 cells or transfected CHO cells expressing derivatives of integrin $\alpha_{IIb}\beta_3$ (21).

‡See ref. 21. NA, not applicable.

Integrin Binding and GAS Pathogenesis. GAS pathogenesis is clearly a complex multifactorial process (1), caused in part by the substantial genetic diversity present among isolates (2–5). Although there is not a unique correlation between the presence of the RGD motif and the ability of an isolate to cause severe invasive disease, we did discover that many of the M types with this motif are abundant causes of severe invasive infections (2, 17, 33). Most noteworthy was the presence of the RGD motif in SpeB2, which is expressed by all serotype M1 isolates. For reasons that are not known, these organisms are the most common cause of invasive disease episodes in the United States and virtually every country surveyed (17). In

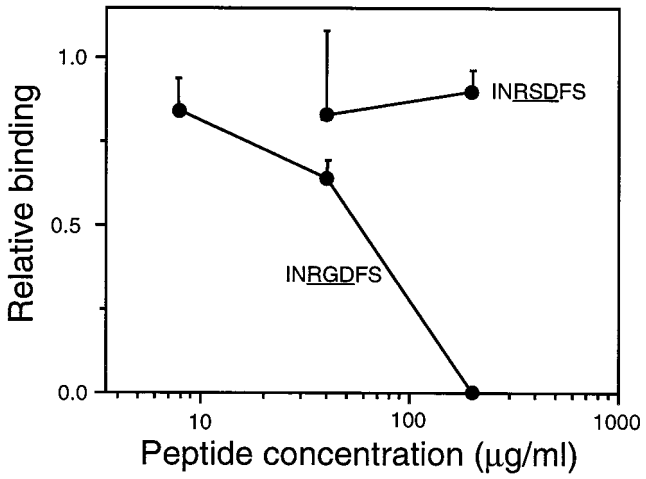


FIG. 3. A peptide encompassing the SpeB2 RGD motif blocks $\alpha_v\beta_3$ binding to SpeB2. 835 cells, which express $\alpha_v\beta_3$, were incubated with the SpeB2 heptapeptide INRGDFS or the corresponding SpeB1 peptide INRSDFS at the indicated concentrations. The cells were added to microtiter wells containing immobilized SpeB2, and after allowing time for binding, unbound cells were removed by washing, and bound cells were quantitated (see *Materials and Methods*). The means and SD of quadruplicate samples are shown.

some countries, M1 strains can account for virtually all episodes of invasive infection caused by GAS (17). Endothelial cell damage and fulminant tissue destruction are two of the hallmarks of severe serotype M1 invasive infections such as necrotizing fasciitis. The molecular mechanism mediating invasion of the human host by serotype M1 GAS is not understood, but serologic studies have documented that SpeB is produced *in vivo* during human invasive episodes (5, 7).

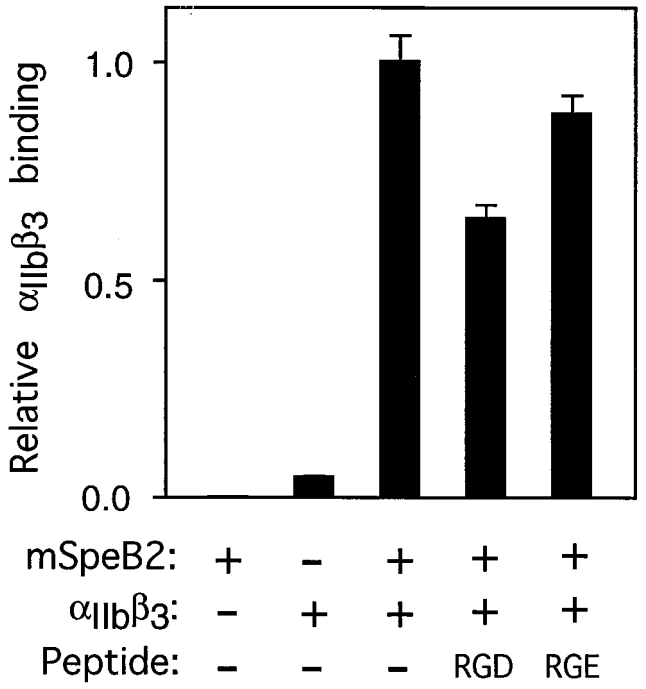


FIG. 4. SpeB binds to purified integrin $\alpha_{IIb}\beta_3$. Microtiter wells were coated or mock-coated with mSpeB2 (2.5 μg/ml) and then probed with purified $\alpha_{IIb}\beta_3$ or buffer control. Stably bound receptor was measured by ELISA (see *Materials and Methods*). The integrin was preincubated with SpeB peptides INRGDFS or INRGEFS (200 μg/ml) for 30 min before binding, as indicated. Binding in the presence of the RGD-containing peptide was significantly less than binding in the presence of the RGE-containing peptide ($P = 0.0024$ by unpaired two-tailed t test). The data are expressed as the means and SD of quadruplicate samples.

Our working hypothesis to explain the role of SpeB in pathogenesis has been dominated by the notion that the protease participates primarily by direct cleavage of host molecules (3, 5, 18). The present data suggest that the situation is far more complex for organisms with the RGD motif in SpeB such as M1 isolates. Integrin binding by mSpeB2 may contribute to GAS pathogenesis by several pathways. Recently we have shown by immunogold electron microscopy that, in addition to occurring free in infected host tissue, SpeB also is located on the GAS cell surface (unpublished work). Hence the RGD motif may participate in bacterial adherence to host cell surfaces and thereby assist in colonization, invasion, and tissue destruction. Another mechanism potentially contributing to pathogenesis is inhibition of platelet aggregation via engagement of integrin $\alpha_{IIb}\beta_3$. In this context, it is noteworthy that, as a population, patients with invasive disease caused by M1 isolates are significantly more likely to have thrombocytopenia and coagulopathy than individuals infected with other M-type isolates (A. McGeer, personal communication).

mSpeB2 is distinct from all other microbial integrin-binding proteins because it is also a potent protease that degrades critical host-cell molecules. Thus, integrin binding might concentrate mSpeB2 at the human cell surface, thereby enhancing the efficiency of local degradation of host molecules such as fibronectin and vitronectin (3) or activation of host matrix metalloproteases (9). The model we favor is based on the fact that mSpeB2 shares obvious functional features with ADAMs (a disintegrin and metalloproteinase), a family of eukaryotic proteins with both integrin binding ("disintegrin") and protease domains (34). One member of the ADAMs family mediates release of tumor necrosis factor α from cell surfaces (35, 36). Interestingly, evidence has been presented that this cytokine participates in GAS pathogenesis (37). Other ADAMs are present in certain snake venoms and are highly potent toxins that disrupt cell-matrix interactions, inhibit platelet aggregation, promote profuse local hemorrhage, and cause death (34). Several of these phenotypes are observed in patients with severe GAS invasive disease.

Regardless of the exact mechanisms involved, integrin binding by mSpeB2 initiates a pathogenic process fundamentally different from integrin engagement by other microbial proteins such as pertactin, filamentous hemagglutinin, or invasin, which apparently serve mainly to initiate colonization or penetration of epithelial cell surfaces (12, 15, 16). Elucidation of the exact events occurring distal to mSpeB2-integrin binding may suggest novel GAS therapies, especially for severe invasive infections that have high mortality, such as those caused by M1 isolates. By showing that a specific variant of a virulence factor interacts in a distinctive fashion with the host, our data add to the important emerging theme in bacterial pathogenesis that allelic variation in virulence genes influences the character of host-pathogen interaction and outcome. Hence, a full analysis of the molecular basis of virulence must consider not only which microbial factors participate, but also the structural variation in these factors present in natural populations.

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