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Is plasminogen deployed as a *Streptococcus pyogenes* virulence factor?

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Abstract

Streptococcus pyogenes (group A streptococcus) causes human skin and throat infections as well as highly invasive diseases including necrotising fasciitis. Group A streptococcal infections and invasive disease have made a resurgence in developed countries over the last two decades. *S. pyogenes* utilise multiple pathways for the acquisition and activation of human plasminogen, securing potent proteolytic activity on the bacterial cell surface. Recent experimental evidence using a humanised transgenic mouse model suggests a critical role for human plasminogen in the dissemination of *S. pyogenes* in vivo.

Keywords

Streptococcus pyogenes, streptococcal infections, plasminogen, CMMB

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Is plasminogen deployed as a *Streptococcus pyogenes* virulence factor?

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Abstract (85 words): *Streptococcus pyogenes* (group A streptococcus) causes human skin and throat infections as well as highly invasive diseases including necrotising fasciitis. Group A streptococcal infections and invasive disease have made a resurgence in developed countries over the last two decades. *S. pyogenes* utilise multiple pathways for the acquisition and activation of human plasminogen, securing potent proteolytic activity on the bacterial cell surface. Recent experimental evidence using a humanised transgenic mouse model suggests a critical role for human plasminogen in the dissemination of *S. pyogenes in vivo*.

Invasive diseases caused by *S. pyogenes*: Group A streptococcus (*Streptococcus pyogenes*) causes a variety of illnesses with diverse clinical manifestations (see text box) [1]. Since the mid-1980's, there has been a global resurgence of severe invasive group A streptococcal infections [2-4]. These diseases progress rapidly and are associated with high rates of morbidity and mortality despite the prompt use of antibiotics. Infection rates have persisted over the last 20 years which has spurred new research into identifying host susceptibility factors and bacterial determinants that may explain these epidemiological changes.

Although no single group A streptococcal virulence determinant has been exclusively associated with a specific disease type, early research identified an association between certain M protein serotypes (i.e. M1 and M3) and invasive infections [5]. Numerous other studies from the 1990's suggested that the resurgence of invasive group A streptococcal infections was due to the emergence of "highly virulent" strains that were clonal in origin [6-11]. Nonetheless, more recent studies examining larger strain sets and using more powerful methodologies to characterise the subtle genetic diversity among isolates have failed to demonstrate a significant association between any particular group A streptococcal clone and invasive infections [12].

S. pyogenes strains of M1T1 and M3 serotypes can be isolated from both uncomplicated infections such as pharyngitis and severe invasive infections such as necrotising fasciitis [5,13]. These group A streptococcal strains can also vary greatly in disease frequency and exhibit epidemic behaviour suggesting these strains are capable of rapid genetic change. Beres et al. [14] found that the major generator of distinct genotypes within 255 group A streptococcal strains (serotype M3) was the acquisition and loss of prophages encoding known and putative virulence factors. This molecular process combined with altered immune recognition due to a four amino acid change in the N-terminal region of the M protein is believed to have significantly contributed to an epidemic wave of M3 invasive infections [14]. Phage sequences present in the two sequenced M3 genomes have also been detected in the genome of *S. pyogenes* M1T1, a strain that is often isolated from invasive diseases [15]. The genetic mosaicism and unique gene sequences present in these phage cassettes act as additional sources of genetic variability and suggest phages can be shared between different M serotypes and even different bacterial species [15]. Host genetic factors have also been shown to play an important role in determining the severity of invasive group A streptococcal infections by altering the magnitude of the cytokine and inflammatory responses against secreted streptococcal pyrogenic exotoxins [16,17].

Common to all invasive group A streptococcal diseases is the requirement of the bacteria to gain entry into areas of the body that are normally sterile sites. Plasminogen binding and its activation to the broad-spectrum serine protease plasmin has been implicated as a contributing mechanism of invasion for *S. pyogenes* and a variety of other bacterial species [18,19].

Binding of plasminogen to the group A streptococcal cell surface: Plasminogen is the multidomain, 791 amino acid circulating zymogen form of plasmin. Plasminogen contains five kringle domains with lysine-binding sites identified in kringles 1, 2, 4 and 5 which are responsible for binding to fibrinogen and plasminogen receptors [20]. Two pathways have been

described in the literature for the binding of plasminogen to the group A streptococcal cell surface. Direct plasminogen binding is mediated by the three known surface plasminogen receptors: plasminogen-binding group A streptococcal M-like protein (PAM or M53; [21]), α -enolase (surface enolase or SEN; [22]) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alternatively known either as plasminogen receptor (Plr) or streptococcal surface dehydrogenase (SDH) [22,23]. Additionally, indirect binding of plasminogen is mediated by the formation of a trimolecular complex consisting of plasminogen, streptokinase and fibrinogen. This complex is then bound to the group A streptococcal cell surface via either plasminogen or fibrinogen receptors [24-26].

Of the three known plasminogen receptors, published evidence suggests that PAM makes the largest contribution to direct plasminogen binding by group A streptococci. In two independent studies, PAM-positive *S. pyogenes* isolates have been found to bind significantly higher levels of plasminogen than PAM-negative isolates [26,27] (Figure 1A). Svensson et al. [27] found an association between PAM-positive isolates and impetigo. Nonetheless, in a study of group A streptococcal isolates from the Northern Territory of Australia where skin infections are endemic, PAM-positive isolates were found to cause bacteremia, throat and skin infections [26]. Binding of PAM to plasminogen is mediated by amino-terminal plasminogen-binding tandem repeat motifs containing lysine residues (designated a1 and a2) in the PAM molecule, which interact with the lysine binding kringle 2 domain of plasminogen [21,28,29].

Plasminogen has been reported to bind to cell-surface located glycolytic enzymes α -enolase and GAPDH [22,23]. Both GAPDH and α -enolase interact with lysine binding kringles domain of plasminogen via carboxy-terminal lysine residues [22,30]. Such carboxy-terminal lysine residues usually bind to kringle 1, 4 and 5 of plasminogen [20]. Whilst α -enolase has been described as a

high affinity plasminogen receptor [22], the failure of PAM-negative isolates to bind significant levels of plasminogen by the direct binding pathway [26,27] suggests that α -enolase may play a more prominent role in the indirect binding pathway by interacting with a trimolecular complex consisting of plasminogen, streptokinase and fibrinogen (Figure 1A). GAPDH has been reported to bind plasminogen with low affinity [22]. Neither GAPDH nor α -enolase contain known secretion signals or membrane anchor motifs and the mechanism by which these glycolytic enzymes become surface exposed is unknown. Both enzymes have also been detected in *S. pyogenes* culture supernatants [31,32]. Other researchers have suggested hypotheses to account for cell-surface GAPDH and α -enolase including the release of such proteins from group A streptococcal cells by autolysis with the released protein potentially binding back to the surface of neighbouring cells or the existence of a hitherto uncharacterised transport mechanism [22,33]. Recently, asymmetric protein secretion of streptococcal pyrogenic exotoxin B (SpeB) was shown to occur at distinct cytoplasmic membrane microdomains termed ExPortals [34]. The role that this structure plays in the secretion of other *S. pyogenes* proteins is currently unknown. However, GAPDH and α -enolase are also asymmetrically distributed on the GAS cell surface [22,33,35], reminiscent of the distribution of SpeB at the GAS ExPortal (Figure 2). We therefore hypothesise that both GAPDH and α -enolase are secreted from the group A streptococcal cell via the ExPortal then subsequently bind to the cell surface.

As mentioned above, the indirect plasminogen-binding pathway in *S. pyogenes* requires streptokinase and fibrinogen [24,25,36] (Figure 1A). Takada and Takada [36] first reported the capacity of plasminogen, fibrinogen and streptokinase to form a trimolecular complex. This complex, which forms extracellularly then binds to the surface of group A streptococci, possesses both plasmin [25] and plasminogen-activator [37] activities. This pathway may

represent the primary plasminogen surface-acquisition mechanism in PAM-negative *S. pyogenes* isolates.

Acquisition of surface plasmin: The conversion of plasminogen to plasmin can be achieved by either the mammalian plasminogen activators urokinase (uPA) and tissue plasminogen activator (tPA), or by secreted microbial activators such as streptokinase [19,20]. As distinct from tPA or uPA, which directly cleave the plasminogen activation bond (i.e. Arg561-Val562) to generate plasmin, streptokinase has no protease activity and cannot directly cleave this bond. Instead, streptokinase forms a 1:1 complex with plasminogen. The preferred catalytic substrate of this complex is the activation loop of other plasminogen molecules [38]. Plasminogen bound to the group A streptococcal surface can also interact with streptokinase leading to bacterium bound plasmin activity [25,37,39]. Additionally, tPA and uPA have been shown to activate plasminogen bound to the group A streptococcal surface [21,40] (Figure 3A). The streptokinase-plasminogen activator complex and surface-bound plasmin cannot be inhibited by host plasmin inhibitors (i.e. α 2-antiplasmin and α 2-macroglobulin) [19,41]. This suggests a propensity of *S. pyogenes* for unregulated plasminogen activation capacity and plasmin activity regardless of host inhibitors.

However, Tewodros et al. [42] found no correlation between secreted streptokinase activity *in vitro* and disease manifestation. Furthermore, high plasminogen binding strains were found to secrete low levels of streptokinase activity [42] (Figure 1B). Phylogenetic analysis of the β -domain region of the gene encoding streptokinase (*ska*) revealed 2 major sequence clusters (clusters 1 and 2) [43]. Most PAM-positive strains possess the subcluster 2b *ska* allele. PAM-negative strains fall into either cluster 1 or subcluster 2a *ska* alleles [43]. Interestingly, PAM-positive strains are high plasminogen binders and acquire streptokinase-dependent and/or tPA-dependent surface plasmin activity (Figure 1C) yet secrete low levels of active streptokinase [39]

(Figure 1B). The lack of streptokinase activity is possibly attributable to the expression of the cysteine protease SpeB in stationary phase cultures which degrades streptokinase [39]. On the other hand, relatively high levels of active streptokinase are secreted by low plasminogen binding strains [42] (Figure 1B). An alternative explanation for these observations may be related to the various *ska* alleles. Kalia and Bessen [43] proposed that subcluster 2b forms of streptokinase have adapted for interaction with plasminogen in the open conformation induced by binding to PAM rather than the closed circulating form of plasminogen (i.e. in solution phase) preferred by the other subcluster forms of streptokinase (Figure 3B and 3C, respectively). Thus, if subcluster 2b forms of streptokinase can only form streptokinase-plasminogen activator complexes when surface bound to PAM, this may account for the apparent lack of streptokinase activity in the supernatants of PAM-positive strains (Figure 1B). Non-cluster 2b forms of streptokinase may interact with both free and surface-bound forms of plasminogen (Figure 3C).

Contribution of plasmin to the dissemination of S. pyogenes: The importance of plasminogen in streptococcal pathogenicity is defined by the biochemical and molecular evolution of streptococcal plasminogen activation. The interaction of streptokinase with plasminogen reflects the mammalian host range of various streptococcal species [44] [45]. The species specificity of plasminogen activation and its importance in virulence are exemplified in murine models of group A streptococcal infection, where murine plasminogen is resistant to activation by *S. pyogenes* streptokinase [46] and mice are generally resistant to skin infection with *S. pyogenes* [47]. Accordingly, a high streptokinase-producing *S. pyogenes* strain and its streptokinase-deletion mutant show no difference in virulence in a mouse skin infection model [48]. In contrast, when a source of human plasminogen is present at the infection site, the presence of the streptokinase gene markedly enhances group A streptococcal virulence [39,48]. Likewise, virulence of streptokinase-producing *S. pyogenes* is enhanced in the mouse by preincubation in human plasma, but not plasminogen-depleted plasma [49].

The recently developed humanised plasminogen transgenic mouse model of infection definitively demonstrated the critical role of human plasminogen in group A streptococcal pathogenicity *in vivo*. The presence of a human plasminogen transgene markedly increased mortality following skin infection with *S. pyogenes*, but not intravenous infection. The susceptibility conferred by the human plasminogen transgene is dependent on streptokinase secretion by *S. pyogenes*, and is characterized by enhanced bacterial dissemination. Fibrinogen may be essential to host defense against group A streptococcal skin infection, possibly due to encapsulation of the bacteria within fibrin networks and prevention of systemic access by occlusion of the local vasculature. Acquisition of fibrinolytic activity by *S. pyogenes* circumvents fibrin-dependent mechanisms, even if only transiently, thereby enhancing virulence [47].

Even in the absence of streptokinase, the ability to focus plasminogen at the group A streptococcal surface via cell-surface receptors enhances virulence *in vivo*. A streptokinase-deficient *S. pyogenes* strain with plasminogen-binding capacity showed enhanced virulence when a human plasminogen source was provided, probably due to plasmin formation by host activators, tPA or uPA [48]. Human plasminogen is more readily activated by murine tPA than is murine plasminogen [46]. The binding of plasminogen to *S. pyogenes* is known to markedly increase the rate of plasminogen activation by tPA [40] supporting the hypothesis that cell-surface binding of plasminogen is an important component of group A streptococcal pathogenesis.

Conclusion: Current knowledge of the mechanisms of plasmin(ogen) binding to the *S. pyogenes* cell surface, the activation of surface acquired plasminogen and the contribution of this protease to the dissemination of group A streptococci *in vivo* suggests plasmin(ogen) plays an important

role in invasive disease progression. Elucidation of the mechanism(s) by which human plasminogen is bound to the group A streptococcal surface and activated may reveal new targets for therapeutic intervention and the control of invasive disease.

References:

- 1 Breiman, R.F., Davis, J.P., Facklam, R., Gray, B.M., Hoge, C.W., Kaplan, E.L., Mortimer, E.A., Schlievert, P.M., Schwartz, B., Stevens, D.L. and Todd, J.K. (1993) Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. The Working Group on Severe Streptococcal Infections. *JAMA* 269 (3), 390-391
- 2 Stevens, D.L. (1996) Invasive group A streptococcal disease. *Infect Agents Dis* 5 (3), 157-166
- 3 Davies, H.D., McGeer, A., Schwartz, B., Green, K., Cann, D., Simor, A.E. and Low, D.E. (1996) Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. *N Engl J Med* 335 (8), 547-554
- 4 Low, D.E., Schwartz, B. and McGeer, A. (1997) The reemergence of severe group A streptococcal disease: an evolutionary perspective. In *Emerging Infections I* (Scheld, W.M. et al., eds.), pp. 93-123, American Society for Microbiology Press
- 5 Johnson, D.R., Stevens, D.L. and Kaplan, E.L. (1992) Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. *J Infect Dis* 166 (2), 374-382

- 6 Musser, J.M., Kapur, V., Szeto, J., Pan, X., Swanson, D.S. and Martin, D.R. (1995) Genetic diversity and relationships among *Streptococcus pyogenes* strains expressing serotype M1 protein: recent intercontinental spread of a subclone causing episodes of invasive disease. *Infect Immun* 63 (3), 994-1003
- 7 Musser, J.M., Kapur, V., Kanjilal, S., Shah, U., Musher, D.M., Barg, N.L., Johnston, K.H., Schlievert, P.M., Henrichsen, J. and Gerlach, D. (1993) Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of *Streptococcus pyogenes* expressing allelic variants of pyrogenic exotoxin A (Scarlet fever toxin). *J Infect Dis* 167 (2), 337-346
- 8 Carapetis, J., Robins-Browne, R., Martin, D., Shelby-James, T. and Hogg, G. (1995) Increasing severity of invasive group A streptococcal disease in Australia: clinical and molecular epidemiological features and identification of a new virulent M-nontypeable clone. *Clin Infect Dis* 21 (5), 1220-1227
- 9 Cleary, P.P., Kaplan, E.L., Handley, J.P., Wlazlo, A., Kim, M.H., Hauser, A.R. and Schlievert, P.M. (1992) Clonal basis for resurgence of serious *Streptococcus pyogenes* disease in the 1980s. *Lancet* 339 (8792), 518-521
- 10 Cockerill, F.R., 3rd, MacDonald, K.L., Thompson, R.L., Roberson, F., Kohner, P.C., Besser-Wiek, J., Manahan, J.M., Musser, J.M., Schlievert, P.M., Talbot, J., Frankfort, B., Steckelberg, J.M., Wilson, W.R. and Osterholm, M.T. (1997) An outbreak of invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children. *JAMA* 277 (1), 38-43

- 11 Cockerill, F.R., 3rd, Schlievert, P.M. and Musser, J.M. (1998) Epidemiology of invasive streptococcal infections. *J Infect Dis* 177 (6), 1773-1774
- 12 Johnson, D.R., Wotton, J.T., Shet, A. and Kaplan, E.L. (2002) A comparison of group A streptococci from invasive and uncomplicated infections: are virulent clones responsible for serious streptococcal infections? *J Infect Dis* 185 (11), 1586-1595
- 13 Chatellier, S., Ihendyane, N., Kansal, R.G., Khambaty, F., Basma, H., Norrby-Teglund, A., Low, D.E., McGeer, A. and Kotb, M. (2000) Genetic relatedness and superantigen expression in group A streptococcus serotype M1 isolates from patients with severe and nonsevere invasive diseases. *Infect Immun* 68 (6), 3523-3534
- 14 Beres, S.B., Sylva, G.L., Sturdevant, D.E., Granville, C.N., Liu, M., Ricklefs, S.M., Whitney, A.R., Parkins, L.D., Hoe, N.P., Adams, G.J., Low, D.E., DeLeo, F.R., McGeer, A. and Musser, J.M. (2004) Genome-wide molecular dissection of serotype M3 group A *Streptococcus* strains causing two epidemics of invasive infections. *Proc Natl Acad Sci USA* 101 (32), 11833-11838
- 15 Aziz, R.K., Edwards, R.A., Taylor, W.W., Low, D.E., McGeer, A. and Kotb, M. (2005) Mosaic Prophages with horizontally acquired genes account for the emergence and diversification of the globally disseminated M1T1 clone of *Streptococcus pyogenes*. *J Bacteriol* In press
- 16 Norrby-Teglund, A., Chatellier, S., Low, D.E., McGeer, A., Green, K. and Kotb, M. (2000) Host variation in cytokine responses to superantigens determine the severity of invasive group A streptococcal infection. *Eur J Immunol* 30 (11), 3247-3255

- 17 Kotb, M., Norrby-Teglund, A., McGeer, A., El-Sherbini, H., Dorak, M.T., Khurshid, A., Green, K., Peeples, J., Wade, J., Thomson, G., Schwartz, B. and Low, D.E. (2002) An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. *Nat Med* 8 (12), 1398-1404
- 18 Lahteenmaki, K., Kuusela, P. and Korhonen, T.K. (2001) Bacterial plasminogen activators and receptors. *FEMS Microbiol Rev* 25 (5), 531-552
- 19 Coleman, J.L. and Benach, J.L. (1999) Use of the plasminogen activation system by microorganisms. *J Lab Clin Med* 134 (6), 567-576
- 20 Ranson, M. and Andronicos, N.M. (2003) Plasminogen binding and cancer: promises and pitfalls. *Front Biosci* 8, s294-304
- 21 Berge, A. and Sjobring, U. (1993) PAM, a novel plasminogen-binding protein from *Streptococcus pyogenes*. *J Biol Chem* 268 (34), 25417-25424
- 22 Pancholi, V. and Fischetti, V.A. (1998) alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. *J Biol Chem* 273 (23), 14503-14515
- 23 Lottenberg, R., Broder, C.C., Boyle, M.D., Kain, S.J., Schroeder, B.L. and Curtiss, R., 3rd. (1992) Cloning, sequence analysis, and expression in *Escherichia coli* of a streptococcal plasmin receptor. *J Bacteriol* 174 (16), 5204-5210
- 24 Wang, H., Lottenberg, R. and Boyle, M.D. (1995) Analysis of the interaction of group A streptococci with fibrinogen, streptokinase and plasminogen. *Microb Pathog* 18 (3), 153-166

- 25 Wang, H., Lottenberg, R. and Boyle, M.D. (1995) A role for fibrinogen in the streptokinase-dependent acquisition of plasmin(ogen) by group A streptococci. *J Infect Dis* 171 (1), 85-92
- 26 McKay, F.C., McArthur, J.D., Sanderson-Smith, M.L., Gardam, S., Currie, B.J., Sriprakash, K.S., Fagan, P.K., Towers, R.J., Batzloff, M.R., Chhatwal, G.S., Ranson, M. and Walker, M.J. (2004) Plasminogen binding by group A streptococcal isolates from a region of hyperendemicity for streptococcal skin infection and a high incidence of invasive infection. *Infect Immun* 72 (1), 364-370
- 27 Svensson, M.D., Sjobring, U. and Bessen, D.E. (1999) Selective distribution of a high-affinity plasminogen-binding site among group A streptococci associated with impetigo. *Infect Immun* 67 (8), 3915-3920
- 28 Wistedt, A.C., Ringdahl, U., Muller-Esterl, W. and Sjobring, U. (1995) Identification of a plasminogen-binding motif in PAM, a bacterial surface protein. *Mol Microbiol* 18 (3), 569-578
- 29 Wistedt, A.C., Kotarsky, H., Marti, D., Ringdahl, U., Castellino, F.J., Schaller, J. and Sjobring, U. (1998) Kringle 2 mediates high affinity binding of plasminogen to an internal sequence in streptococcal surface protein PAM. *J Biol Chem* 273 (38), 24420-24424
- 30 Winram, S.B. and Lottenberg, R. (1998) Site-directed mutagenesis of streptococcal plasmin receptor protein (Plr) identifies the C-terminal Lys334 as essential for plasmin binding, but mutation of the *plr* gene does not reduce plasmin binding to group A streptococci. *Microbiology* 144, 2025-2035

- 31 Lei, B., Mackie, S., Lukomski, S. and Musser, J.M. (2000) Identification and immunogenicity of group A Streptococcus culture supernatant proteins. *Infect Immun* 68 (12), 6807-6818
- 32 Aziz, R.K., Pabst, M.J., Jeng, A., Kansal, R., Low, D.E., Nizet, V. and Kotb, M. (2004) Invasive M1T1 group A Streptococcus undergoes a phase-shift in vivo to prevent proteolytic degradation of multiple virulence factors by SpeB. *Mol Microbiol* 51 (1), 123-134
- 33 Pancholi, V. and Chhatwal, G.S. (2003) Housekeeping enzymes as virulence factors for pathogens. *Int J Med Microbiol* 293 (6), 391-401
- 34 Rosch, J. and Caparon, M. (2004) A microdomain for protein secretion in Gram-positive bacteria. *Science* 304 (5676), 1513-1515
- 35 Pancholi, V. and Fischetti, V.A. (1992) A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. *J Exp Med* 176 (2), 415-426
- 36 Takada, Y. and Takada, A. (1989) Evidence for the formation of a trimolecular complex between streptokinase, plasminogen and fibrinogen. *Thromb Res* 53 (4), 409-415
- 37 D'Costa, S.S. and Boyle, M.D. (1998) Interaction of a group A Streptococcus within human plasma results in assembly of a surface plasminogen activator that contributes to occupancy of surface plasmin-binding structures. *Microb Pathog* 24 (6), 341-349

- 38 Parry, M.A., Zhang, X.C. and Bode, I. (2000) Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. *Trends Biochem Sci* 25 (2), 53-59
- 39 Svensson, M.D., Sjobring, U., Luo, F. and Bessen, D.E. (2002) Roles of the plasminogen activator streptokinase and the plasminogen-associated M protein in an experimental model for streptococcal impetigo. *Microbiology* 148 (Pt 12), 3933-3945
- 40 Kuusela, P., Ullberg, M., Saksela, O. and Kronvall, G. (1992) Tissue-type plasminogen activator-mediated activation of plasminogen on the surface of group A, C, and G streptococci. *Infect Immun* 60 (1), 196-201
- 41 Lottenberg, R., Minning-Wenz, D. and Boyle, M.D. (1994) Capturing host plasmin(ogen): a common mechanism for invasive pathogens? *Trends Microbiol* 2 (1), 20-24
- 42 Tewodros, W., Norgren, M. and Kronvall, G. (1995) Streptokinase activity among group A streptococci in relation to streptokinase genotype, plasminogen binding, and disease manifestations. *Microb Pathog* 18 (1), 53-65
- 43 Kalia, A. and Bessen, D.E. (2004) Natural selection and evolution of streptococcal virulence genes involved in tissue-specific adaptations. *J Bacteriol* 186 (1), 110-121
- 44 Boyle, M.D. and Lottenberg, R. (1997) Plasminogen activation by invasive human pathogens. *Thromb Haemost* 77 (1), 1-10
- 45 Gladysheva, I.P., Turner, R.B., Sazonova, I.Y., Liu, L. and Reed, G.L. (2003) Coevolutionary patterns in plasminogen activation. *Proc Natl Acad Sci U S A* 100 (16), 9168-9172

- 46 Lijnen, H.R., Bachmann, F., Collen, D., Ellis, V., Pannekoek, H., Rijken, D.C. and Thorsen, S. (1994) Mechanisms of plasminogen activation. *J Intern Med* 236 (4), 415-424
- 47 Sun, H., Ringdahl, U., Homeister, J., Fay, W.P., Engleberg, C., Yang, A., Rozek, X., Wang, L., Sjobring, U. and Ginsberg, D. (2004) Plasminogen is a critical host pathogenicity factor for group A streptococcal infection. *Science* 305, 1283-1286
- 48 Khil, J., Im, M., Heath, A., Ringdahl, U., Mundada, L., Cary Engleberg, N. and Fay, W.P. (2003) Plasminogen enhances virulence of group A streptococci by streptokinase-dependent and streptokinase-independent mechanisms. *J Infect Dis* 188 (4), 497-505
- 49 Li, Z., Ploplis, V.A., French, E.L. and Boyle, M.D. (1999) Interaction between group A streptococci and the plasmin(ogen) system promotes virulence in a mouse skin infection model. *J Infect Dis* 179 (4), 907-914
- 50 Loy, J.A., Lin, X., Schenone, M., Castellino, F.J., Zhang, X.C. and Tang, J. (2001) Domain interactions between streptokinase and human plasminogen. *Biochemistry* 40 (48), 14686-14695

Figures and Text Box

Figure 1. Plasminogen binding and activation by PAM-positive and PAM-negative *S. pyogenes* isolates. (A) Group A streptococcal binding of 125 I-plasminogen alone (hatched bars) or in the presence of excess streptokinase and fibrinogen (filled bars; data from [26]). (B) Streptokinase activity in THY culture supernatants ($A_{600} = 1.1$). Similar data were obtained at $A_{600} = 0.6$ (results not shown). (C) Group A streptococcal cell-surface plasmin activity following 3h incubation in plasma (filled bars) or plasminogen-depleted plasma (hatched bars). Streptokinase alleles for each strain were as follows: NS53, subcluster 1; NS13, subcluster 2b; NS32, subcluster 2b; NS931, subcluster 1; NS730, subcluster 1; NS474, subcluster 1. All measurements represent mean (\pm SD) of triplicate determinations.

Figure 2. Immuno-electron microscopy revealing the asymmetric distribution of SpeB [34], SEN [22,33] and GAPDH [33,35] on the GAS cell surface. Clusters of immuno-gold particles are boxed.

Figure 3. Schematic diagram summarising the possible pathways of cell surface plasmin acquisition by various *S. pyogenes* strains. Plasminogen is a single chain 791 amino acid glycoprotein containing seven distinct structural domains, which are designated the amino-terminal peptide (NTP) followed by Kringles 1–5 (K1-5) and the carboxy terminal serine protease domain (PD). K1, K2, K4 and K5 contain lysine-binding motifs that are responsible for binding to fibrinogen and to plasminogen receptors. Lysine-dependent interaction/s between the NTP and K5 are also responsible for maintaining the circulating zymogen in a mostly closed, activation-resistant form. As summarised here, the efficient conversion of plasminogen to its activated twin-chain form plasmin (depicted by loss of the NTP and by the green coloured PD linked to the kringle domains via a disulphide bond shown in red) by mammalian or microbial activators requires a binding-induced conformational change in plasminogen to an open, activation-susceptible form [20,38,50].

(A). Plasminogen bound directly to the cell surface via PAM, or other plasminogen receptors (PL-R), or indirectly via fibrinogen receptors (F-R; not shown) may be activated to plasmin by host activators. (B) Streptokinase contains three structural domains designated the α , β and γ domains, all of which can bind to the PD resulting in the streptokinase-plasminogen activator complex. Generally PAM negative strains secrete subcluster 1 or 2a allele forms of streptokinase [43] which can readily interact with solution phase (free), closed plasminogen (via K5; [50]). This results in a free activator complex that can convert other free plasminogen molecules to plasmin that can then readily bind to the cell surface directly via plasmin(ogen) receptors (e.g. SEN, GAPDH). Alternatively, the activator complex can bind to fibrinogen to form the fibrinogen-streptokinase-plasminogen trimolecular complex. This can then bind to the cell surface via fibrinogen receptors and act on either directly or indirectly bound plasminogen leading to cell surface plasmin. (C) Most PAM-positive strains secrete subcluster 2b allele forms of streptokinase (hatched) which preferentially interact with an open conformation of plasminogen that is induced by binding to PAM via K2. The PAM-bound activator complex can then either activate free plasminogen to plasmin that can readily bind to the cell surface (via PAM and/or other receptors not shown here), or other PAM-bound plasminogen to plasmin. In both cases, this results in cell surface bound plasmin. Although not shown here, the PAM-bound activator complex could also act on indirectly bound plasminogen (via fibrinogen receptors). In all scenarios, bound plasmin is resistant to inhibition by α 2-antiplasmin. In addition, free or PAM-bound streptokinase-plasminogen activator complex, as well as the trimolecular activator complex, are resistant to inhibition by α 2-antiplasmin.

Text Box:

Classification of group A streptococcal infection.

- I. Streptococcal toxic shock syndrome (STSS): Defined as a definite case by isolation of group A streptococci from a normally sterile site (eg. blood cerebrospinal, pleural or peritoneal fluid, tissue biopsy, surgical wound etc.) or a probable case if isolated from a non-sterile site (eg. Throat, sputum, vagina, superficial skin lesion etc.) in conjunction with combinations of the following signs of clinical severity; hypotension, renal impairment, coagulopathy, increased liver activity, adult respiratory distress syndrome, generalised erythematous rash or soft-tissue necrosis.
- II. Other invasive infections: Defined by isolation of group A streptococci from a normally sterile site in patients not meeting criteria for STSS
 - a. Bacteremia with no identified focus
 - b. Focal infections with or without bacteremia. Includes meningitis, pneumonia, peritonitis, puerperal sepsis, osteomyelitis, septic arthritis, necrotizing fasciitis, surgical wound infections, erysipelas and cellulitis.
- III. Scarlet Fever: Defined by a scarletina rash with evidence of group A streptococcal infection, most commonly pharyngotonsillitis.
- IV. Non-invasive infections: Defined by the isolation of group A streptococci from a non-sterile site.
 - a. Mucous membrane: includes pharyngitis, tonsillitis, otitis media, sinusitis, vaginitis
 - b. Cutaneous: includes impetigo.
- V. Non-suppurative sequelae: Defined by specific clinical findings with evidence of a recent group A streptococcal infection
 - a. Acute rheumatic fever
 - b. Acute glomerulonephritis

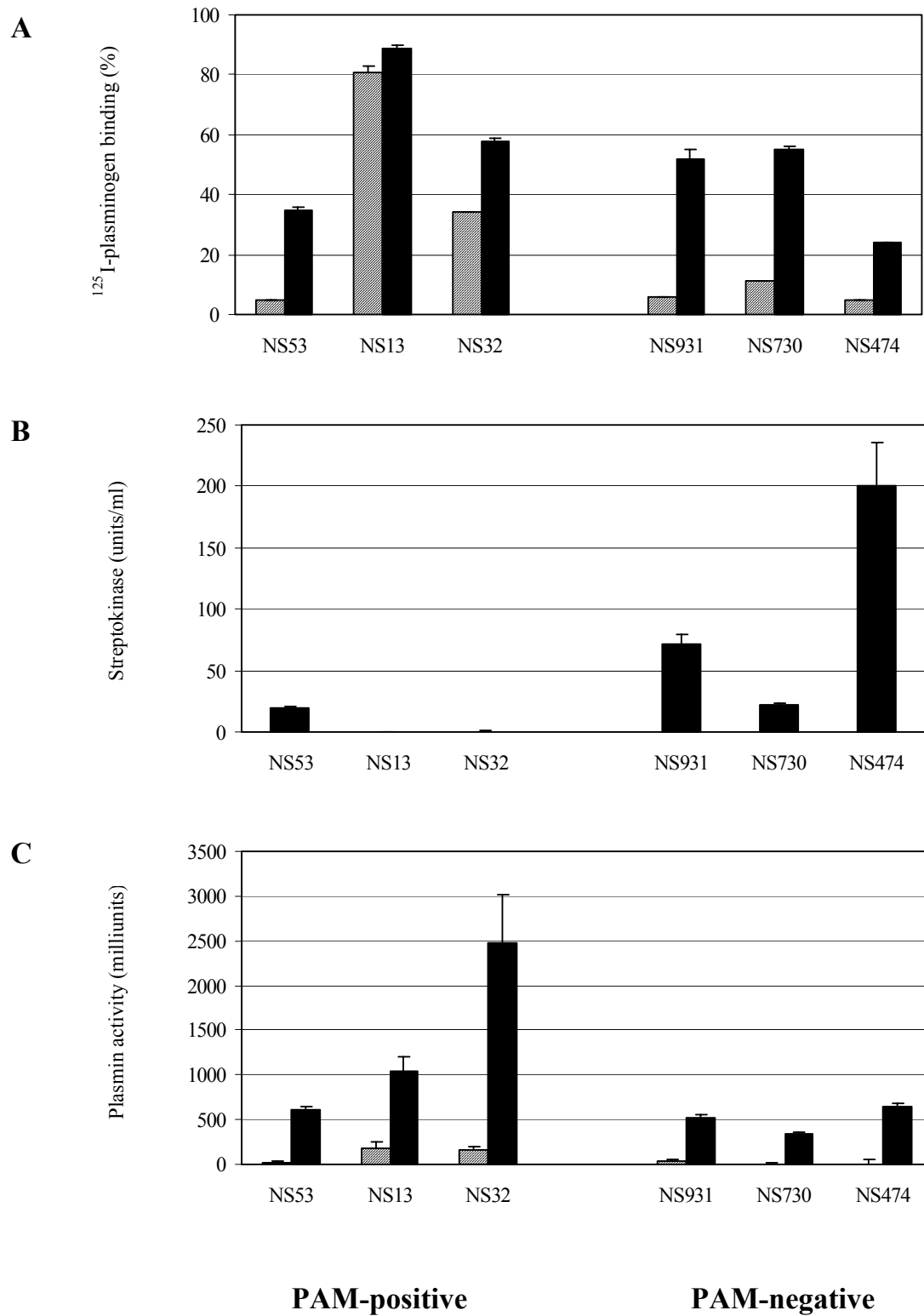


Figure 1.

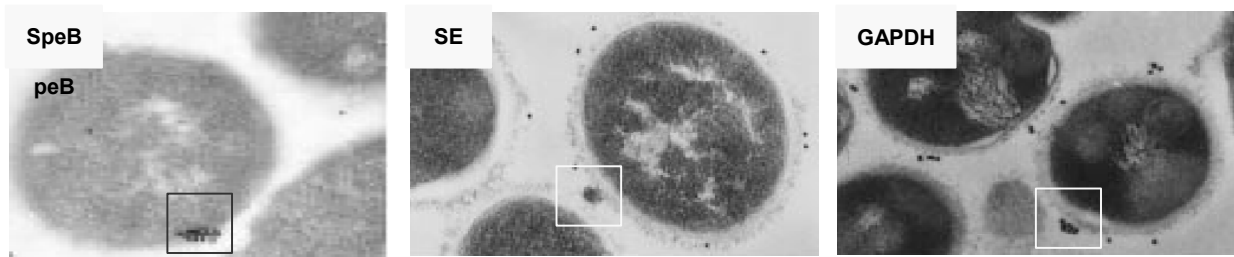


Figure 2.

