1-3-2012

The role of streptokinase as a virulence determinant of streptococcus pyogenes - potential for therapeutic targeting

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Streptococcus pyogenes is a major human pathogen responsible for numerous diseases ranging from uncomplicated skin and throat infections to severe, life threatening invasive disease such as necrotising fasciitis and streptococcal toxic shock syndrome. These severe invasive infections progress rapidly and produce high rates of morbidity and mortality despite the implementation of aggressive treatment plans. The activation of plasminogen and the acquisition of plasmin activity at the bacterial cell surface is critical for the invasive pathogenesis of this organism. To facilitate this process, S. pyogenes secrete streptokinase, a potent plasminogen activating protein. Here, we describe the role of streptokinase in invasive pathogenesis and discuss some potentially useful strategies for disruption of streptokinase mediated plasminogen activation which could be employed to treat severe invasive S. pyogenes infections.

Keywords

pyogenes, targeting, streptococcus, therapeutic, determinant, virulence, streptokinase, role, potential, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

McArthur, J. D., Cook, S. M., Venturini, C. & Walker, M. J. (2012). The role of streptokinase as a virulence determinant of streptococcus pyogenes - potential for therapeutic targeting. Current Drug Targets, 13 (3), 297-307.

Title: The role of streptokinase as a virulence determinant of *Streptococcus pyogenes* – potential for therapeutic targeting.

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Abstract

Streptococcus pyogenes is a major human pathogen responsible for numerous diseases ranging from uncomplicated skin and throat infections to severe, life threatening invasive disease such as necrotising fasciitis and streptococcal toxic shock syndrome. These severe invasive infections progress rapidly and produce high rates of morbidity and mortality despite the implementation of aggressive treatment plans. The activation of plasminogen and the acquisition of plasmin activity at the bacterial cell surface is critical for the invasive pathogenesis of this organism. To facilitate this process, *S. pyogenes* secrete streptokinase, a potent plasminogen activating protein. Here, we describe the role of streptokinase in invasive pathogenesis and discuss some potentially useful strategies that disrupt streptokinase mediated plasminogen activation and could be employed to treat severe invasive *S. pyogenes* infections.

Streptococcus pyogenes Infections

Streptococcus pyogenes (Group A streptococcus; GAS) is a Gram-positive, β-hemolytic human pathogen associated with diverse infections ranging from asymptomatic mild respiratory and skin ailments such as pharyngitis and impetigo to life-threatening forms of invasive disease such as necrotising fasciitis and streptococcal toxic shock syndrome. Superficial self-limiting infection of the upper-respiratory tract is the most common disease manifestation of GAS infection. Systemic diseases arise from the capacity of GAS to degrade and cross epidermal and mucosal barriers and invade deep subcutaneous tissues. Although invasive GAS disease is much less common, its significance remains high due to the rapidity of spread and severity of symptoms [1-3]. The overall burden of GAS disease worldwide is of significant concern [2,4]. Acute post-streptococcal glomerulonephritis and acute rheumatic fever are immunologically mediated post-streptococcal non-suppurative sequelae that often follow repeated GAS infection and are a cause of morbidity and mortality in children and young adults worldwide [5,6].

GAS disease can be successfully treated by use of antimicrobial agents and since the introduction of penicillin in the 1940s, rates of GAS infections declined over time until the mid-1980s when a resurgence of invasive infections was observed. Outbreaks of rheumatic fever, toxic shock syndrome and severe skin infections have been reported worldwide in the past three decades [5-9]. There is an established non-random relationship between certain serotypes and streptococcal disease type [2,5,8,10,11], and even though host genetic/immune predisposition plays an equally important role in the emergence of severe invasive disease in an individual [12,13], evolution of bacterial pathogenic potential seems to have driven this epidemiological change.

M1, M3 and M18 types in particular have shown a strong association with invasive disease cases in the USA and Europe [6,9,14-16] while in indigenous Australian populations the correlation between severe disease and specific M types is less defined and more complex [4,17]. In particular the M1T1 serotype is the most frequently isolated GAS type in clinical cases worldwide and has been associated both with non-invasive and invasive disease [11,18,19]. Due to the acquisition of novel

lysogenic bacteriophages, this globally disseminated M1T1 clone contains in its genome a unique array of virulence determinants in comparison to those found in strains of the M1 serotype with which M1T1 shares its chromosomal content. The M1T1 clone carries the bacteriophage-encoded *speA* and *sda1* virulence genes but not the *speC* gene found in the genome of M1 GAS strain SF370 [18,20-22].

S. pyogenes virulence factors

GAS possess many putative and defined virulence factors implicated in disease development, including both extracellular proteins and surface-exposed molecules that play a role in adherence to host tissues, in host immune system evasion and in bacterial dissemination. Several reviews describing in detail the proven and hypothesized functions of GAS virulence determinants and their role in disease (see [2,5,6,9,23-25]).

At the onset of infection GAS strains colonize the skin and soft-tissues by attaching to the host epithelial cells via the interaction of several streptococcal adhesins and specific host receptors [5]. Colonization is mostly extracellular although cellular invasion by *S. pyogenes* has been documented [26,27]. Persistence within the host is then mediated by virulence factors involved in mechanisms of immune system evasion such as disruption or inhibition of complement activation, opsonisation and phagocytosis, and degradation of immunoglobulins as well as other host proteins [5,23]. These virulence determinants include the hyaluronic acid capsule, M and M-like proteins, cytotoxins, lysins (SLO), proteases (SpeB; IdeS), DNases (Sda) and several secreted streptococcal pyrogenic exotoxins (Spes). The Spes are potent microbial stimulants of the immune system also known as superantigens [5,6,28,29].

Because *S. pyogenes* is highly adapted to colonize various niches in its human host, the expression of virulence genes is tightly regulated in response to growth phase and changing environmental cues during infection. The Mga regulon along with the RofA-like proteins, Rgg and several two-component regulatory systems including the two-component control of virulence regulatory sensor kinase operon (CovR/S), control the level of transcription of most streptococcal virulence determinants [30].

Mechanisms of invasive disease

Invasive GAS disease such as necrotising fasciitis requires bacterial entry into areas of the human body that are normally sterile through degradation of internal host tissue barriers. A number of secreted GAS proteins potentially play a role in this process by degrading DNA, hyaluronic acid deposits, and host proteins [2,5,31]. GAS produces several streptodornases, proteins with DNase activity, that are thought to be involved in GAS pathogenesis by participating in the clearing of pus at the site of local infection and consequently aiding invasion [5,32] and by playing a role in evasion of the host immune system through degradation of neutrophil extracellular DNA traps [33,34]. Most known streptococcal DNases (types A, C and D) are phage-encoded proteins [32,35].

The broad-spectrum cysteine protease SpeB is an extracellular protein secreted as a 40 kDa zymogen that is proteolitically cleaved to its 28 kDa active form. It is found in all GAS strains but differentially secreted [5]. SpeB is responsible for cleaving cytokine precursors, immunoglobulins, cell receptors and ECM proteins [36-38]. In accordance with these properties, inactivation of SpeB in some murine models of infection resulted in decreased lethality and reduced dissemination to organs highlighting a key role of SpeB activity in GAS virulence [5,39-41].

The interaction of GAS with human plasminogen

The exploitation of host components to increase the chance of survival and spread within the human host is an essential invasive pathogenic mechanism of GAS. Human plasminogen is often used by invasive bacteria as a virulence factor and this process has been recognized as a critical step in GAS invasion [42-46]. In the healthy host, active plasmin dissolves intravascular fibrin clots and participates in the repair and remodelling of tissue by activating host extracellular metalloproteases and collagenases [47]. These same mechanisms are exploited by GAS to promote systemic spread [44,46,48,49].

Plasmin(ogen) can be bound directly to the GAS cell surface via three known receptors, plasminogen-binding group A streptococcal M-like proteins (PAM or Prp)

[50,51], α-enolase (surface enolase or SEN) [52] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alternatively known either as plasminogen receptor (Plr) or streptococcal surface dehydrogenase (SDH) [53,54]. Additionally, plasminogen may be indirectly bound to the cell surface through the formation of a trimolecular complex consisting of plasminogen, streptokinase and fibrinogen which may bind via either plasminogen or fibrinogen receptors [55-57]. Plasminogen bound to the bacterial cell surface may also be converted into plasmin through the action of the host derived plasminogen activators urokinase (u-PA) and tissue plasminogen activator (t-PA) [58], or by the activity of the GAS plasminogen activator streptokinase (SK). SK is a highly efficient plasminogen activator that plays a critical role in the invasive pathogenesis of GAS [43,49]. Studies on this protein have highlighted the species-specificity of the interaction between GAS streptokinase and the human plasminogen activation system [31,46]. Streptokinase production in the presence of human plasminogen has been shown to enhance GAS virulence in murine models of skin infection [44,45,59].

Streptokinase; a bacterial plasminogen activator

Plasminogen is the 791-amino-acid zymogen form of the human serine protease plasmin. Plasminogen contains several structural domains, consisting of the amino-terminal peptide, followed by five kringle domains (K1–K5) and the carboxy-terminal serine protease catalytic domain [60]. Lysine-binding sites present in K1, K4, and K5 are responsible for the interaction of plasminogen with a number of other molecules such as fibrin(ogen), plasminogen receptors and other cellular surfaces [60]. The circulating, soluble form of plasminogen (Glu-plasminogen) is maintained in a compact, closed conformation through lysine-dependent interactions between the amino-terminal peptide and kringles K4 and K5 [61,62]. On binding to mammalian or bacterial receptors, a conformational change is induced in Glu-plasminogen, producing an extended, activation-susceptible form [63]. Cleavage of the Lys77-Lys78 peptide bond in Glu-plasminogen by plasmin removes the amino-terminal peptide domain producing Lys-plasminogen which also displays an extended open conformation that is more readily activated [64].

A range of bacterial pathogens are capable of interacting with human plasminogen (see Table 1) [43,65]. This interaction can contribute to the course of an infection directly through protease mediated degradation of tissue barriers or by affecting the immune response being generated by the host. In *Staphylococcus aureus*, which secret the plasminogen activating protein called staphylokinase, plasmin activity localised on the bacterial cell surface will cleave deposited C3b and IgG from the cell thereby inhibiting complement function and opsonisation through a reduction in phagocytosis by neutrophils and macrophages [66]. Similarly, *S. pyogenes* isolates are capable of acquiring plasmin activity onto the cell surface but how this effects the immune response is yet to be examined [67].

Streptokinase is a 414-residue, plasminogen activating protein secreted by many β-haemolytic streptococcal species [68]. Streptokinase is composed of three structural domains; α (aa 1 to 150), β (aa 151 to 287) and γ (aa 288 to 414) [69]. Each domain has a similar structure and displays a typical β grasp fold consisting of a major β sheet composed of 5 β strands and an α helix in the α and β domains and 4 β strands and a coiled coil segment instead of the α helix in the γ domain [69]. Unlike the human plasminogen activators, u-PA and t-PA, which cleave the plasminogen activation bond (Arg561-Val562) to generate plasmin, streptokinase lacks intrinsic protease activity. Instead, streptokinase forms a stoichiometric complex with plasminogen and through non-proteolytic mechanisms, generates an active site in the bound plasminogen molecule to produce an activator complex [70,71]. Upon binding of streptokinase to plasminogen, a conformational rearrangement occurs where the N-terminal Ile^{1} residue is inserted into the N-terminal binding cleft of plasminogen allowing this residue to form a salt bridge with Asp740 of plasminogen and triggering the formation of the active site [72,73]. Unlike plasmin formed by the proteolytic activation of plasminogen, the activator complex can bind to substrate plasminogen and cleave the activation bond to produce plasmin [74]. As streptokinase has a much higher affinity for plasmin, plasminogen present in the activator complex will readily exchange with free plasmin producing a complex that that will readily activate other free plasminogen molecules [75]. The activity displayed by both plasminogen and plasmin containing activator complexes is not inhibited by the major mammalian plasmin regulators, α_2-antiplasmin and α_2-macroglobulin [70,76].

Streptokinase alleles of *S. pyogenes* isolates exhibit considerable genetic diversity [77] and form two distinct phylogenetic lineages (cluster 1 and cluster 2) [78,79]. Streptokinase variation has been linked to strains associated with acute post streptococcal glomerulonephritis [80,81] and to strains that exhibit skin tissue tropism [78]. However, the biological significance of streptokinase polymorphism in streptococcal pathogenesis has not been determined. It has recently been shown that variant streptokinase proteins produced by distinct clinical *S. pyogenes* isolates display differing plasminogen activation capacities [79]. Streptokinase encoded by cluster 1 *ska* alleles readily formed an active complex with soluble plasminogen while cluster 2 streptokinase required the formation of a trimolecular complex with plasminogen and fibrinogen to display plasminogen-activating capability [79]. The phenotypic differences displayed by streptokinase variants suggest that these proteins may have differing roles in streptococcal pathogenesis.

Despite such phenotypic differences, mechanisms have been proposed by which all *S. pyogenes* strains with differing *ska* alleles can acquire plasmin activity onto the cell surface. Plasminogen receptors interact with different regions of the plasminogen protein. PAM binds to plasminogen *via* interaction with K2 [82], whereas fibrinogen and other streptococcal plasminogen receptors (SEN and GAPDH) bind *via* lysine-dependent interactions with K1, K4, and K5 [60]. Therefore, in strains that express cluster 2 type streptokinase, the active trimolecular complex can only be bound to the cell surface via PAM or fibrinogen-binding receptors (FgR) such as M1 (Figure 1A). Alternatively, strains that express cluster 1 type streptokinase, which do not require fibrinogen to form an active complex with plasminogen, can bind the activator complex directly to the bacterial cell surface *via* plasminogen receptors (PLRs) or through an interaction with fibrinogen and fibrinogen receptors (Figure 1B) [79]. Streptokinase polymorphism and the functional differences displayed by these proteins may need to be considered when designing inhibitors that target streptokinase mediated plasminogen activation for therapeutic purposes.

Streptokinase as a thrombolytic therapeutic

Occlusion of blood vessels by thrombus (blood clot) is an essential function of haemostasis. During healthy haemostatic function, thrombosis is tightly controlled.

In the event of vascular injury, thrombosis is rapidly initiated to prevent blood loss. Thrombosis also functions as a defence mechanism to prevent systemic dissemination of invasive bacteria by encapsulation in fibrin networks [83]. In the event of pathological conditions and/or failure to maintain haemostasis, several cardiovascular disease states can arise and are classified into three main categories: atherosclerotic heart disease (acute myocardial infarction), cerebrovascular disease (stroke) and venous thromboembolism (deep-vein thrombosis and pulmonary embolism) [84-86]. Globally, the incidence of cardiovascular disease is increasing and represents a major cause of disability and mortality [84,87].

There are numerous strategies available for the treatment of thrombosis. A common approach is thrombolytic therapy which involves the use plasminogen activators to dissolve clots, resulting in reperfusion of the occluded blood vessels and improved outcomes from disease states [84]. The therapeutic potential of the bacterial plasminogen activator, streptokinase, has long been recognised and this activator was first trialled as a treatment for acute myocardial infarction in 1958 [88]. Streptokinase was first approved by the US Food and Drug Administration (FDA) for the treatment of acute myocardial infarction in the mid 1980's and is now used for the treatment of myocardial infarction, pulmonary embolism, deep vein thrombosis, arteriovenous-cannula occlusions and peripheral arterial occlusions [89].

The relative cost effectiveness of streptokinase as a fibrinolytic agent has made streptokinase one of the most widely used thrombolytic therapeutics [90]. Although streptokinase is the most efficient activator of human plasminogen [91], its fibrin independent activation mechanism limits the effectiveness of streptokinase as a therapeutic. When administered intravenously, streptokinase will form an activator complex with circulating plasminogen which will subsequently begin to generate plasmin in the circulation at sites distant to that of occluding clots. This explosive plasmin generation depletes circulating levels of fibrinogen, plasminogen and factors V and VIII producing systemic fibrinolysis and a hypocoagulable state, which in turn, can lead to non-specific bleeding events. High levels of circulating plasmin also increases the production of bradykinin which leads to a lowering of blood pressure [92-94]. As streptokinase is of bacterial origin, it is highly immunogenic, which can reduce the effective half-life of the protein in the circulation and can limit the number

of times streptokinase can be used to treat a patient [95]. Therefore, in developed countries the use of streptokinase has declined in favour of therapeutics based on the human plasminogen activators, u-PA and t-PA.

Both u-PA and t-PA are trypsin like serine proteases which activate plasminogen directly and are non-immunogenic (Table 2). u-PA activates both circulating and fibrin bound plasminogen, but does not bind to fibrin itself. u-PA has a half-life of 15-20 min which results in less systemic fibrinolysis when compared to streptokinase [96]. In developed countries, t-PA is the most common fibrinolytic agent used for treatment of coronary artery thrombosis, pulmonary embolism and acute stroke [89]. t-PA is a fibrin specific plasminogen activator which means plasmin production is confined to the area of thrombus. Circulating t-PA is rapidly cleared from plasma (initial half-life of 4-10 min) resulting in high rates of re-thrombosis [93]. As a result, for greatest efficacy t-PA administration is via an initial bolus injection, followed by short continuous infusion [93]. Such large doses can produce systemic fibrinolysis and unwanted bleeding episodes, side effects similar to those seen with streptokinase treatment. Therefore, much research has been aimed at improving the efficacy of current plasminogen activators or identifying novel activators with more favourable plasminogen activation characteristics, some of which have been summarised in Table 2.

Second generation thrombolytic therapeutics based on streptokinase

Streptokinase is an immunogenic protein and as humans are frequently exposed to streptococcal infections, the presence of circulating anti-streptokinase antibodies can be detected in most individuals [97]. Upon exposure to purified streptokinase protein during thrombolytic therapy, high titres of anti-streptokinase antibodies are generated and can be long lasting (up to 54 months) [95]. High levels of circulating anti-streptokinase antibodies reduce the effectiveness of repeat streptokinase therapy by rapidly neutralising streptokinase upon administration or by causing numerous allergic complications [98,99]. A number of antigenic regions have been identified within the streptokinase protein by using murine monoclonal antibodies or patient sera [100-103]. Targeting these regions with deletion or site directed mutagenesis has been proposed as possible mechanisms by which the immunogenicity of streptokinase

can be reduced. An obvious requirement with this approach is to retain the plasminogen activating properties of native streptokinase. Parhami-Seren *et al.* [104] used naturally occurring variants of streptokinase from different strains of group G and group A streptococcus to determine how amino acid sequence variation in previously identified antigenic epitope regions affected antibody binding and streptokinase function. In this study, variant streptokinase proteins which contained a Ser138Lys substitution were not recognised by an anti-streptokinase monoclonal antibody but displayed normal plasminogen activation activity. When this amino acid change was introduced into therapeutic streptokinase, the mutant protein displayed unchanged plasminogen activation activity but was not bound by the anti-streptokinase mAb [104]. Streptokinase variation in GAS has evolved through the interaction of this pathogen with the human fibrinolytic and immune systems. Therefore, careful analysis of novel variants in future studies will assist in identifying regions that can be mutated for immunogenic purposes without disrupting plasminogen activation function.

Attempts to increase the *in vivo* half-life of streptokinase have utilised chemical modifications and site directed mutagenesis. Plasmin will cleave streptokinase at Lys59 and Lys386 producing a 37 kDa intermediate which retains only 16% of the plasminogen activation activity of native streptokinase [105]. A Lys59Glu streptokinase mutant maintained plasminogen activation activity that was comparable to native streptokinase but displayed increased resistance to plasmin proteolysis indicating that this variant may have a longer functional half-life in therapeutic applications [106]. Similarly, PEGylated streptokinase proteins also maintain good activator function but are protected from proteolytic degradation and display decreased antigenicity due to increased steric interference [107,108]. Currently, the only modified streptokinase approved for human use is acylated plasminogen-streptokinase activator complex (APSAC; antistreplase) [93]. This therapeutic consists of an inactive complex of streptokinase and Lys-plasminogen containing an acylated catalytic centre. Upon injection, APSAC undergoes a controlled deacylation before displaying thrombolytic activity which serves to increase the half-life of this therapeutic [109]. This allows APSAC to be administered as a rapid, bolus injection which is more favourable when compared with the long infusions required with native

streptokinase. However, the higher costs associated with this therapy has limited the use of APSAC in clinical settings [93] (Table 2).

Modifying the plasminogen activation characteristics of streptokinase has also been attempted to improve the therapeutic potential of this molecule. Fibrin-independent plasminogen activation by the streptokinase-plasminogen activator complex is thought to be due to the insensitivity of the activator complex to α_2-antiplasmin inhibition and due to the ability of the activator complex to activate circulating Glu-plasminogen in the absence of fibrin [110]. Critical for these characteristics is the α-domain of streptokinase. Streptokinase mutants harbouring an N-terminal, 59 amino acid deletion (SKΔ59) of the α-domain will only form an activator complex with plasmin which is susceptible to α_2-antiplasmin inhibition and is a poor activator of circulating Glu-plasminogen [111,112]. However, the SKΔ59-plasmin complex will readily activate unfolded forms of plasminogen such as Glu-plasminogen in the presence of fibrin [111]. When compared to t-PA, this mutated form of streptokinase displays less fibrinogen degradation in human plasma and has a greater capacity to degrade blood clots *in vivo* using a murine model of human thrombosis [110].

Novel therapeutics for the treatment of invasive *S. pyogenes* infections

Severe invasive GAS infections include bacteraemia, necrotizing fasciitis and streptococcal toxic shock syndrome. Estimates suggest there are over 660,000 cases of invasive GAS infections per year which results in over 160,000 deaths world wide [4]. Streptococcal toxic shock syndrome is characterised by hypotension and multiple organ failure and may be accompanied by necrotizing fasciitis which is a progressive bacterial infection of the skin, subcutaneous tissue and the underlying fascia. The rapidity by which these invasive infections can progress produces high rates of mortality (30-70%) despite the implementation of aggressive treatment plans [113]. Recommended treatment consists of surgical debridement, fluid replacement/blood pressure support and high-dose intravenous antibiotic therapy (commonly penicillin combined with clindamycin to reduce bacterial production of pyrogenic exotoxins and superantigens) [114]. More recently the successful use of polyclonal human intravenous immunoglobulin (IVIG) for the treatment of invasive infections has been

demonstrated in numerous *in vitro* studies [12,115-118]. However, the use of IVIG as an adjunctive treatment in a clinical setting has been less promising [119,120]. Therefore, there is a need to develop additional therapeutics that may complement current treatments and can restrict the systemic spread of *S. pyogenes* during the early stages of infection.

Plasminogen activation and subsequent acquisition of protease activity onto the cell surface of *S. pyogenes* is critical for invasive disease initiation. Therefore, the possibility exists to target the bacterial factors involved in this process as a means to prevent systemic infection. One possible strategy would be to inhibit streptokinase mediated plasminogen activation without affecting physiological plasminogen activation mechanisms. This could be achieved by designing specific inhibitors that (1) prevent the initial interaction between streptokinase and plasminogen (2) prevent the streptokinase mediated conformational rearrangement of plasminogen that exposes the active site or (3) prevent the plasminogen-streptokinase activator complex from binding substrate plasminogen.

The majority of physiological interactions involving plasminogen are mediated through lysine binding sites located within the various kringle domains. This includes binding to a variety of ligands such as fibrin(ogen), α_2-antiplasmin, histidine rich glycoprotein, thrombospondin and cellular receptors [60]. Streptokinase binds to Glu-plasminogen, Lys-plasminogen and plasmin with increasing affinities displaying dissociation constants of 130 nM, 10 nM and 12 pM respectively [72,75]. The increasing affinity for Lys-plasminogen and plasmin is due to increased lysine binding sites interactions which occur as the conformation of plasmin(ogen) becomes more open and extended making lysine binding sites within kringle domains more accessible for interaction with the C-terminal Lys414 residue of streptokinase [121]. However, initial binding of streptokinase to the compact closed conformation of Glu-plasminogen is independent of lysine binding sites [72,122]. As Glu-plasminogen is the most prevalent circulating form of plasminogen, inhibiting the lysine independent interaction between streptokinase and Glu-plasminogen may represent a potential strategy for preventing streptokinase binding to plasminogen with minimal disruption to the physiological function(s) of plasmin(ogen).

As described earlier, streptokinase mediated plasminogen activation involves the formation of an activator complex where streptokinase binds to the catalytic domain of plasminogen and induces a conformational rearrangement that activates the catalytic site non-proteolytically. However, the majority of plasmin is generated proteolytically by the activator complex converting free plasminogen into plasmin. This is achieved through the formation of a substrate recognition exosite in the activator complex which mediates the specific recognition of substrate plasminogen for proteolytic activation [123]. The α domain of streptokinase contributes to the formation of this exosite and is critical for the activator complex to capture, extend and process substrate Glu-plasminogen [112,124]. Specifically inhibiting streptokinase α domain interactions would therefore hinder streptokinase mediated plasminogen activation through numerous mechanisms. Such a strategy could prevent non-proteolytic Glu-plasminogen activation in the activator complex and/or could prevent the activator complex acting on substrate Glu-plasminogen molecules resulting in an overall reduction in the production of plasmin activity.

Conclusion

Streptokinase is an important streptococcal virulence factor which has a critical role in the pathogenesis of invasive streptococcal diseases. The ability of streptokinase to activate host plasminogen to the broad spectrum protease plasmin facilitates the acquisition of plasmin activity onto the bacterial cell surface. Surface bound plasmin activity promotes systemic spread of bacterial cells through degradation of tissue barriers and through modulation of the host immune response. Inhibiting this critical process by designing specific inhibitors or using antibodies that interfere with streptokinase mediated plasminogen activation may help to halt the rapid progression of invasive *S. pyogenes* infections and thus reduce the high morbidity and mortality associated with these diseases.

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Table 1. Bacterial pathogens that interact with human plasminogen.

Pathogen	**Plasminogen binding**	**Plasminogen activation**	**Reference**
Bacillus anthracis	Y		[125]
Bifidobacteria Spp.	Y		[126]
Borrelia burgdorferi	Y		[127]
Escherichia coli	Y		[128]
Francisella tularensis	Y		[129]
Haemophilus influenzae	Y		[130]
Helicobacter pylori	Y		[131]
Legionella pneumophila	Y	Y	[132]
Leptospira Spp.	Y		[133]
Listeria monocytogenes	Y		[134]
Moraxella catarrhalis	Y		[130]
Mycobacterium tuberculosis	Y		[135]
Mycoplasma fermentans	Y		[136]
Neisseria Spp	Y		[137]
Proteus mirabilis	Y		[130]
Pseudomonas aeruginosa	Y		[138]
Salmonella enteritidis	Y		[128]
Staphylococcus aureus	Y	Y	[139]
Streptococcus pneumoniae	Y		[140]
Streptococcus Spp (group A, C and G)	Y	Y	[141]
Trichomonas vaginalis	Y		[142]
Yersinia pestis	Y	Y	[143]

Table 2: Comparison of thrombolytic agents that currently approved or being developed for human use.

	Streptokinase*	Antistreplase (APSAC)*	u-PA*	Pro-u-PA	t-PA (Alteplase)*	rt-PA (Reteplase)*	TNK-PA (Tenecteplase)*	nPA	Staphylokinase
Molecular Weight (kDa)	47	131	32/55	49	68	39.5	65	53.5	16.5
Source	*Streptococcus equisimilis*	*Streptococcus equisimilis*, plasminogen anisoylated	Recombinant, human fetal, kidney	Recombinant, *E. coli* or, mammalian cells	Recombinant, human melanoma cell line	Recombinant, human mutant t-PA	Recombinant plus mutation	Recombinant plus mutation	*Staphyloccocus aureus*
Plasminogen Activation	Indirect	Indirect	Direct	Direct	Direct	Direct	Direct	Direct	Indirect
Half-life (min)	20	100	<5	9	5	14	25	57	6
Fibrin Specificity	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
PAI-1 Resistance	N/A	N/A	No	No	No	No	Yes	No	N/A
Elimination	Hepatic	Hepatic	Renal	Hepatic	Hepatic	Renal	Renal	Hepatic	Hepatic
Antigenic	Yes	Yes	No	No	No	No	No	No	Yes
Dosing (AMI treatment)	1 h infusion	10 min single bolus	1 h infusion	Bolus, 1 hr infusion	Bolus, 90 min infusion	Double bolus	Single bolus	Single bolus	Double bolus over 30 min
Hospital Cost/Dose ($US)	$300	$1,800	$2,000	>$2,000	$2,200	$2,200	$2,200	$2,200	N/A

Abbreviations: kDa = KiloDalton, PAI-1 = Plasminogen Activator Inhibitor-1, u-PA = urokinase, t-PA = tissue type plasminogen activator, N/A = Data Not Available, AMI = acute myocardial infarction, * = FDA approved. [89,93,94,96,144-146]

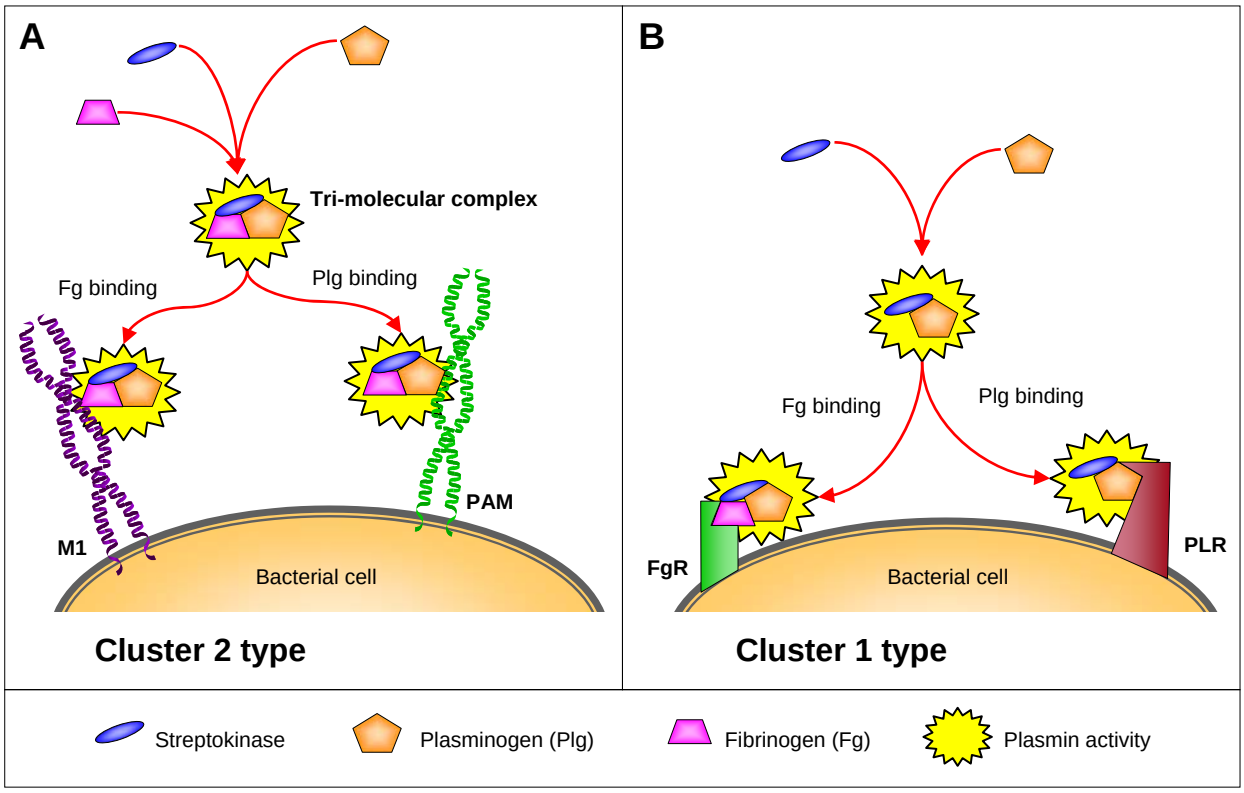
A
Tri-molecular complex
Fg binding
Plg binding
PAM
M1
Bacterial cell
Cluster 2 type
B
Fg binding
Plg binding
FgR
PLR
Bacterial cell
Cluster 1 type
Streptokinase
Plasminogen (Plg)
Fibrinogen (Fg)
Plasmin activity

Figure 1. Schematic diagram summarizing the hypothesized pathways of cell surface plasmin acquisition by GAS strains expressing different alleles of streptokinase. A) Cluster 2 streptokinase must combine with plasminogen and fibrinogen to form a trimolecular complex that exhibits plasmin activity. The trimolecular complex is bound to the cell surface *via* the interaction between PAM and kringle 2 or *via* the interaction between fibrinogen-binding receptors (FgR) such as M1 protein and fibrinogen. Other GAS plasminogen-binding proteins such as SEN cannot bind this trimolecular complex, because the domains K1, K4, and K5 required for interaction are involved in the interaction with fibrinogen. *B*) Cluster 1-type streptokinase will combine with plasminogen to form a complex with plasmin activity. This complex can be bound directly to the bacterial cell surface *via* plasminogen receptors (PLRs) or through an interaction with fibrinogen and fibrinogen receptors (modified from McArthur *et al.*[79]).