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Review Article

Bacterial Plasminogen Receptors Utilize Host Plasminogen System for Effective Invasion and Dissemination

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In order for invasive pathogens to migrate beyond the site of infection, host physiological barriers such as the extracellular matrix, the basement membrane, and encapsulating fibrin network must be degraded. To circumvent these impediments, proteolytic enzymes facilitate the dissemination of the microorganism. Recruitment of host proteases to the bacterial surface represents a particularly effective mechanism for enhancing invasiveness. Plasmin is a broad spectrum serine protease that degrades fibrin, extracellular matrices, and connective tissue. A large number of pathogens express plasminogen receptors which immobilize plasmin(ogen) on the bacterial surface. Surface-bound plasminogen is then activated by plasminogen activators to plasmin through limited proteolysis thus triggering the development of a proteolytic surface on the bacteria and eventually assisting the spread of bacteria. The host hemostatic system plays an important role in systemic infection. The interplay between hemostatic processes such as coagulation and fibrinolysis and the inflammatory response constitutes essential components of host defense and bacterial invasion. The goal of this paper is to highlight mechanisms whereby pathogenic bacteria, by engaging surface receptors, utilize and exploit the host plasminogen and fibrinolytic system for the successful dissemination within the host.

1. Introduction

Bacterial invasion is generally mediated by the bacterial surface and secreted products which often function to circumvent host innate and acquired defense systems. Evasion of host immune response and production of invasive molecules are often critical first steps for initiating systemic diseases. The host hemostatic system plays an important role in systemic infection and bacterial pathogenesis. Hemostatic processes such as coagulation and fibrin deposition, as a result of inflammation, are an essential part of the host defense system. Invasive bacterial pathogens, however, have developed a variety of strategies to elude the host line of defense and gain entry into the surrounding host tissue. The ability to degrade tissue barriers formed by extracellular matrices (ECM) and basement membranes (BM) is one of the most important factors in the pathogenesis of bacterial infection. Degradation of this network by secreted bacterial proteases leads to tissue and structural damage and thereby enhances bacterial invasiveness into the host body. However, a number of invasive bacteria like *Streptococcus*, *Haemophilus, Neisseria* and most enteric bacteria like *E. coli* are extracellular pathogens and produce low levels of proteases. Consequently, degradation and penetration through this network of membranes require the use of different mechanisms for invasion. A number of these mechanisms rely on the interaction with protease-dependent cascade systems of their host which include fibrinolysis, coagulation, phagocytosis, and complement activation. The mammalian fibrinolytic system which constitutes dissolution of thrombus by the serine proteinase plasmin (Pm) offers a potential proteolytic system that could be utilized by pathogenic bacteria to gain entry into the host system. Plasminogen (Plg) binding to bacteria can almost be considered a universal event [1–3]. Plasmin has been known to play a significant role in several physiological processes apart from degradation of fibrin clot in fibrinolysis and various extracellular matrix and connective tissue components like

laminin and fibronectin. Pm also activates procollagenases to active collagenases and is involved in activation of certain prohormones and growth factors [4–6]. Recently, it has also been shown that blood-brain barrier invasion is enhanced by Pm acquisition [7]. Through activation of matrix metalloproteases (MMPs), Pm can break down extracellular matrices and basement membranes either directly or indirectly and degrade complement and immunoglobulins thereby facilitating the likelihood of bacterial spread (see review [3]) [8, 9]. Therefore, Pm activity must be tightly controlled in order to maintain tissue homeostasis and avoid random tissue damage. Such regulation is achieved by the plasminogen system due to the availability of Plg receptors (PlgRs) and plasminogen activators (PAs).

Bacteria interact with the Plg system by secreting PAs and expressing PlgRs on their surface which direct the Pm activity to locations where proteolytic activity is required. Many of the bacterial PlgRs are critical virulence factors and are among the major targets of vaccine development.

The fibrinolytic system is known to play an important role in the inflammatory response to bacterial infections and host Plg system plays a central role in fibrinolysis. The fibrinolytic system functions to break down the existing fibrin-containing blood clot and is an important constituent of wound-healing mechanisms. Fibrin clots are formed during coagulation and injury to blood vessel walls resulting in fibrin deposition and platelet aggregation. The main active enzyme involved in the fibrinolytic process is Pm. Fibrinolysis is initiated when Plg is converted to Pm by host physiological activators urokinase-type plasminogen activator (uPA) or tissue-type plasminogen activator (tPA). During infection, at the site of local microbial injury, host inflammatory cells in association with bacterial secreted products like endotoxins generate a vigorous response in the surrounding vasculature resulting in local vascular thrombosis. This serves to wall off the site of infection and can, in turn, entrap bacteria and acts as a barrier to prevent bacterial invasion and systemic spread. Most likely this is why fibrin deposits are often seen at the site of infection. The formation of active Pm on the bacterial surface facilitates the degradation of the fibrin layer deposited by the host around the site of local infection promoting release of bacteria from fibrin clot and subsequently assists in ECM degradation thereby facilitating the bacterial dissemination into deeper tissues. Therefore, there is an exquisite mechanistic relationship between the bacterial proteins and host protein which facilitate bacterial dissemination and survival. Through their ability to bind to host Plg directly, PlgRs therefore serve to enhance the activation of surface bound Plg to Pm, creating a proteolytic microenvironment to be employed by pathogens to catalyze degradation of matrix barriers, which then assists the spread of the bacteria [23, 24] (Figure 1). In recent years, studies to understand the mechanisms and the role of the Plg system in bacterial infections have increased significantly, and this paper summarizes our present knowledge of a select group of bacterial pathogens that utilize host Plg/Pm system for the dissemination into surrounding tissue.

2. Key Players in the Plasminogen System

2.1. Plasminogen and Plasmin. Human plasminogen (hPlg) is synthesized in the liver as a 90 kDa, 810 amino acid polypeptide chain. During secretion, a 19 amino acid leader peptide is cleaved generating the mature form of this protein, which comprises 791 amino acids [25, 26]. This native form of Plg is called Glu-Plg due to the presence of an aminoterminal glutamic acid residue. The Plg molecule contains a total of seven structural domains, each with different properties. The N-terminal portion of the molecule consists of an activation peptide (AP) followed by a series of 5 repeating homologous triple-disulfide-linked peptide regions, approximately 80 amino acids in length, termed kringles (K1–K5). Cleavage of the peptide bond between residues 77 and 78 is required for the release of the activation peptide. The truncated form of the zymogens is then designated as Lys-Plg. The cleavage of the Arg561-Val562 peptide bond in hPlg leads to the formation of Pm, which contains a heavy chain of 561 amino acid residues, disulfide linked to a light chain of 230 amino acid residues. The 65-kDa heavy chain comprises the N-terminal portion of the Plg molecule, which consists of AP and 5 kringles (K1–K5) (Figure 2). The function of the five kringles in the heavy chain of Plg is primarily to mediate protein-protein interactions, such as those between binding of Plg or Pm to fibrin, ECM targets, and lysine-containing receptors/analogues. Plg kringles are also independently involved in angiogenesis and inhibition of cell migration [27, 28]. The Pm light chain contains the carboxyl terminus of hPlg, which comprises the catalytic domain that resembles that of the serine protease family. The catalytic triad of amino acids that define serine proteases is present in human Pm and consists of His603, Asp646, and Ser741 [5]. The catalytic domain or serine protease domain (SP) of Pm is a compact module that can recruit adapter molecules, or cofactors, such as the bacterial PAs streptokinase and staphylokinase, which modify the substrate presentation to the enzyme and its specificity [29]. Almost all kringle modules bind to lysine or lysine-like ligands except K3. K1 and K4 exhibit the strongest ligand affinities [30–33] while K2 possesses the weakest affinity [34]. K2 shows strong affinity to a endopolypeptide (VEK-30) derived from *Streptococcal* Plg receptor M protein (PAM) [35]. The binding strength of kringle modules is dependent on the nature of the ligand.

Glu-Plg can adopt two different conformations, T and R, which highly influence their activation capability. The T state (tight conformation) is a compact state seen in full-length hPlg. In this conformational state, Plg is poorly activated [36, 37]. Investigations of recombinant (r) Glu-Plg variants have shown that both negative and positive effector molecules have an influence on the structure and activation of Plg [38, 39]. For example, it has been suggested that in the presence of Cl^-, Lys side chains, and/or pseudo-Lys arrangements in the 77 amino acid NH2-terminal AP interact with ω-amino acid binding sites of K1Plg, K4Plg, and to a lesser extent, K5Plg. In addition, studies have shown that a number of activation peptide residues interact with these kringle sites and participate in the stabilization of the

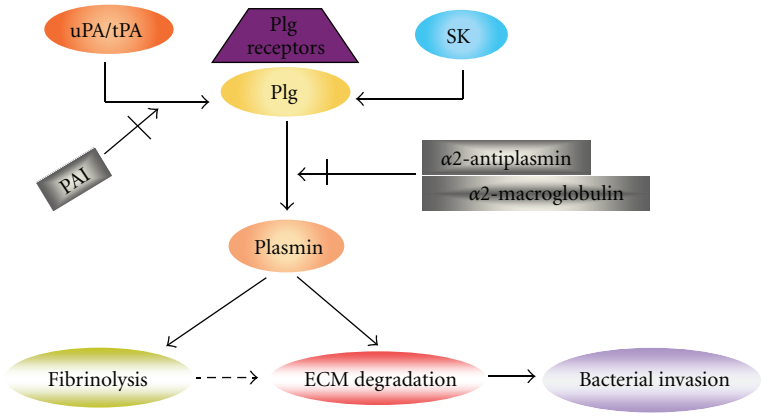

Figure 1: Simplified overview of the mammalian plasminogen system and its utilization by bacteria. Bacterial plasminogen receptors immobilize plasminogen on the surface, which enhances activation of plasminogen to the active serine protease, plasmin, by host plasminogen activators uPA, tPA and/or bacterial plasminogen activators like streptokinase (SK). Both tPA and uPA can be inhibited by plasminogen activator inhibitors (PAI), while plasmin activity is controlled by its major inhibitor, α2-antiplasmin, and to a lesser extent by α2-macroglobulin. Plasmin degrades fibrin clot (fibrinolysis) and various ECM components which enables bacterial migration through tissue barriers.

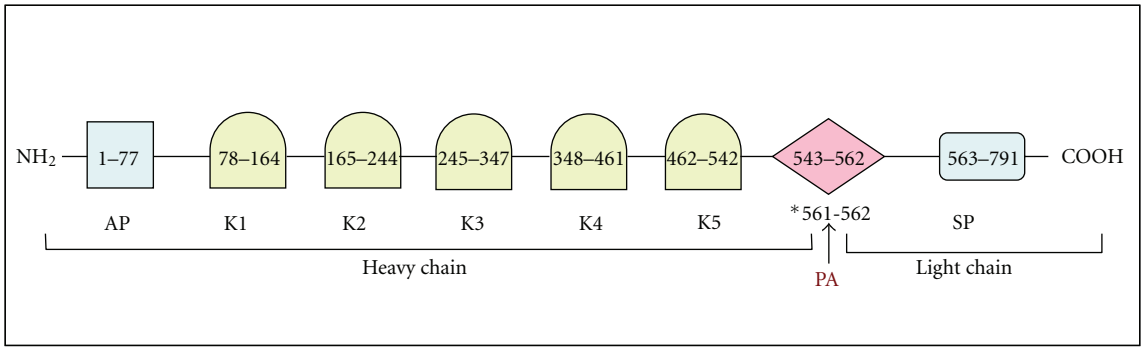

Figure 2: Schematic presentation of the structure of human Plg. From the amino terminus of the mature protein, the 77-residue activation peptide (AP) is followed by 5 kringle domains (K1–K5) containing lysine binding sites and the catalytic SP domain. R561-V562, which is proteolytically cleaved to generate Pm, is shown with an asterisk. The cleavage site for plasminogen activator (PA) is shown by an arrow. Heavy chain and light chains after proteolytic cleavage are marked with lines.

T conformation [40]. On the other hand, the conformation of Glu-Plg changes to a more flexible relaxed state (R state), that is readily activated when the activation peptide (AP) is displaced from its kringle binding sites upon addition of ω-amino acids, for example, ε-aminocaproic acid (EACA). Once Glu-Pm is formed, it acts as a catalyst for cleavage of the AP from Glu-Plg and Glu-Pm, thus catalyzing conversion of Glu-Plg to Lys78-Plg or Glu-Pm to Lys78-Pm. Release of the AP renders Lys78-Plg a more activatable substrate due to its transformation to the R state and transition to a protein that is now insensitive to negative and positive effector molecules [41, 42]. The Lys-Plg conformation enables it to interact with PlgRs and also facilitates ready conversion to Pm on the cell surface [43].

The atomic structures of the kringles as well as a truncated form consisting of a 20 amino acid long fragment of the heavy chain connected to the light chain by two disulfide bridges have been reported [44–47]. The structural changes that the catalytic domain undergoes in association with the activation of Plg have also been identified by crystallography [48].

Recently, the crystal structure of full-length type II human Plg has been solved [49]. The structure shows a closed conformation which reveals that N-terminal AP domain makes extensive contacts with K4 and K5. These interactions, together with SP domain and chloride ions, maintain the T conformation. The Arg561-Val562 peptide bond which is proteolytically cleaved to convert Plg to Pm is shielded by the position of K3/K4 linker which perhaps protects the proenzyme from unwanted activation in the closed state. Position of K3 might also serve as a physical barrier to activating proteases. However, superpositioning SK, which can readily activate the closed state of plasminogen, to the Plg structure revealed that despite these safeguards SK can bind to the SP domain in a way that can cleverly avoid the kringle modules. K3 forms a significant interface (including a disulphide bond) with K2, as previously observed in the structure of angiostatin [50]. These interactions position

K3 next to the SP domain. The authors also noted that differences in glycosylation most likely alter the position of K3. The structure shows that LBS of K1 is exposed and most likely mediates proenzyme recruitment to fibrin or receptors. Previously it was suggested from the structures of isolated kringle domains in the presence or absence of lysine analogs that K1 is most likely not involved in structural rearrangement but another kringle(s) might be. Further analysis of the Plg structure reveals that indeed K5 is the kringle crucial for triggering Plg conformational change and peeling away of K5 from AP domain may initiate that change. Another human plasminogen closed conformation structure published recently also supports these observations and shows that only K1-LBS is readily available for ligand and/or receptor binding while LBS of other kringles, except K1, are involved in intramolecular interactions [51].

2.2. Host Plasminogen Activators. Plasminogen is present in large amount in blood as well as in tissues. Due to the broad biological significance of Plg and Pm in cellular processes, it is therefore of utmost importance that this enormous reservoir of proteolytic activity be tightly regulated. This is achieved by specific PAs, inhibitors, and receptors in order to restrict the generation of Pm, as well as immobilize Plg to its receptors or target molecules.

Mammals have two PAs, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) which were originally identified in tissue and urine extracts, respectively [52]. They recognize and cleave the R560-V561 peptide bond in Plg generating active Pm. uPA has been identified as a critical trigger for Pm generation during cell migration and invasion, under physiological and pathological conditions and, therefore, appears to be the primary PA in eukaryotic cell migration processes, whereas tPA is the main PA in fibrinolysis and maintenance of vascular patency [4]. The activities of PAs are regulated by specific plasminogen activator inhibitors (PAIs) thereby restricting the generation of Pm for extracellular matrix (ECM) as well as intravascular fibrin degradation [53, 54].

Both PAs are serine proteases that are secreted as low-activity single-chain zymogens and proteolytically processed by plasmin into fully active two-chain molecules held together by a single disulfide bond [52, 55, 56]. Crystal structures of the catalytic domains of tPA and uPA have shown that the active sites are located in the B chain in both PAs and Plg specificity is determined by the insertion loops which are positioned around the active site cleft [57, 58]. In uPA, the A chain consists of one kringle structure and a growth factor-like domain, which contains the receptor-binding amino acid sequence [59]. In tPA, the N-terminal region contains a "fibronectin-type II" finger domain, an "EGF-like" domain and two "kringle" domains. The finger-like domain, together with the second kringle domain, provides tPA with high affinity for fibrin [60]. A large serine protease catalytic domain is located at the carboxyl terminus, similar to uPA.

2.2.1. Urokinase-Type Plasminogen Activator (uPA). uPA is expressed in a variety of tissues as a 411 amino acid glycoprotein with an apparent molecular weight of 53 kDa. uPA-mediated Pm generation results in the proteolytic activation of matrix metalloproteinases (MMP), other ECM proteinases, and growth factors. Therefore, uPA initiates a proteolytic cascade that leads to degradation of the ECM, facilitating processes such as cell attachment and detachment, tissue remodeling, and tumor metastasis. uPA binds to a specific glycoprotein receptor (uPAR) which increases its overall catalytic efficiency [61, 62]. uPAR restricts uPA activity to the cell surface, where Plg is also bound to its cognate sites [63]. Plg and PAs are thus colocalized on cells and tissues thereby reinforcing cell-associated proteolysis. uPA/uPAR interactions account for localized pericellular proteolysis, as opposed to the extracellular and plasma proteolytic activity of tPA. Alternatively, uPA-uPAR binding induces a conformational change in the complex, which initiates signal transduction cascades that affect cell proliferation and survival, cytoskeleton dynamics, and cell adhesion [64].

2.2.2. Tissue-Type Plasminogen Activator (tPA). tPA, a 530 amino acid glycoprotein is primarily secreted by vascular endothelial cells as a prozymogen with very low level activity until proteolytically activated by Pm [65]. A variety of stimuli, such as thrombin, histamine, bradykinin, adrenaline, acetylcholine, and shear stress can govern the release of tPA. The major role of tPA is to degrade fibrin in blood vessels. tPA activity is greatly stimulated by fibrin which interacts with kringle 2 and the finger and EGF-like domains [66]. Accordingly, tPA synthesis is induced under ischemic conditions. In tPA-deficient ($tPA^{-/-}$) mice, clot lysis is strongly impaired whereas, in uPA-deficient mice, there is occasional fibrin deposition [67]. In addition to its role in fibrinolysis, tPA has been shown to have an additional role that is unrelated to its proteolytic activity and independent of Pm generation. tPA can act as a ligand to cell surface proteins and trigger several cellular responses [68, 69]. In $tPA^{-/-}$ mice, cerebellar granule neurons migrate significantly slower than granule neurons from wild-type mice; as a consequence, late arriving neurons are impaired in their synaptic interactions [70].

Annexin 2 has been identified as a plasma membrane receptors for tPA in endothelial cells which also possess binding affinity for Plg but not uPA [71–73]. Annexin 2 is a highly conserved member of the annexin super family of calcium-dependent, phospholipid-binding proteins [74]. Like all annexin family members, annexin 2 has a variable N-terminal "tail" and a conserved C-terminal "core" region. Annexin 2 heterotetramer consists of two annexin 2 monomers dynamically linked by two molecules of p11 (also known as S100A10), a member of the S100 family of calcium-binding proteins. Binding of p11 to annexin 2 is essentially irreversible. This heterotetrameric complex which is the predominant species in most cells is referred to as AIIt and provides a surface for tPA and plasminogen interaction and may even have a greater stimulatory effects on tPA-dependent plasmin generation [75, 76]. Both plasminogen and tPA bind

to distinct annexin 2 domains. As discussed earlier, binding of plasminogen to its cellular receptor is dependent on the interaction of plasminogen with the carboxyl-terminal lysines of the receptor. In a purified-protein system, native human placental annexin 2 conferred an approximately 60-fold increase in catalytic efficiency of tPA-dependent Plg activation which is almost completely negated in the presence of Lys analogues indicating a lysine-dependent interaction [75]. As annexin 2 lacks carboxyl-terminal lysine, it has been proposed that proteolytic processing of annexin 2 at a specific site might expose a carboxyl-terminal lysine, specifically Lys307 which could mediate annexin 2 binding to Plg [73]. However, this has never been demonstrated either *in vitro* or *in vivo*. Mutational study of this lysine did not dramatically alter the stimulatory activity of annexin 2 when compared with the wild-type recombinant annexin 2 [75]. On the other hand, p11 subunit of AIIt has the prerequisite carboxyl-terminal lysines, mutation of which resulted in reduced tPA-dependent Plg activation compared to wild-type p11 subunit [75]. These results suggest that carboxyl-terminal lysine residues of p11 mediate Plg binding and it is probably these lysines which result in the stimulation of tPA-dependent Plg activation of AIIt (reviewed in [77]). P11 subunit binds to the aminoterminal side of annexin 2. It is also the aminoterminal in particular Leu-Cys-Lys-Leu-Ser-Leu hexapeptide within the aminoterminal "tail" domain which mediates tPA binding and Cys^8 of this sequence has been proposed to be essential for the binding [78]. Several studies also suggest a physiological role for annexin 2 in fibrin homeostasis. Annexin 2 and p11 are not only involved in regulation of plasmin generation, fibrin homeostasis, and angiogenesis but are also involved in tumor growth, invasion, and metastasis (reviewed in [79]) [80]. Annexin 2 and p11 could therefore play the role of physiological receptors of plasminogen.

Low-density lipoprotein receptor-related protein (LRP) is a large two-chain scavenger receptor that mediates clearance of tPA-PAI-1 complex, *in vitro* [81, 82]. Both growth factor and the finger domain of tPA are required for this interaction. LRP binds a variety of biologically diverse ligands including the broad spectrum protease inhibitor α2-macroglobulin (α2M) and free and PAI-complexed plasminogen activators [83]. LRP sequesters proteases and/or protease-inhibitor complexes and thereby regulates extracellular proteolysis thus decreasing the overall protease load in the pericellular space [84, 85].

Apart from uPA and tPA, certain proteases belonging to coagulation cascade have also been shown to activate Plg directly. These accessory activators include kallikrein, factor XIa and factor XIIa [86, 87]. Type IV collagen is also known to immobilize Plg and lead to enhanced tPA-mediated Plg activation [88]. Factor VII-activating protease has also been reported to be a potent *in vitro* activator of single-chain Plg activators, but its physiological role is not yet clear [89]. Plasma protein histidine-proline-rich glycoprotein (HPRG) also contains carboxyl-terminal lysines and is known to bind Plg [90, 91].

2.3. Plasmin and Plasminogen Activator Inhibitors. The activation of Plg is negatively modulated by a family of serine protease inhibitors known as serpins [92]. The mode of action of serpin requires formation of a stable complex with active site serine of the target protease, followed by the proteolytic cleavage of the serpin by target protease [93].

The chief serpin PA inhibitors are plasminogen activator inhibitor-1, -2, -3 (PAI-1, PAI-2 and PAI-3) and a broad-spectrum protease inhibitor nexin [94]. PAI-3 can inhibit both uPA and tPA albeit with a much slower rate than PAI-1 and -2 [56]. Out of the two major PAIs, PAI-1 is the most ubiquitous and most rapidly acting physiological inhibitor of both uPA and tPA. PAI-2, on the other hand, is less effective towards single-chain tPA and does not inhibit single-chain uPA but can inhibit both two-chain uPA and two-chain tPA with comparable efficiency.

The chief physiological inhibitor of plasmin is the serpin α2-antiplasmin. This glycoprotein forms a complex with plasmin by binding to kringle 1–3 of plasmin [95]. Since the same lysine-binding kringle sites mediate Plg binding to receptors, receptor-bound plasmin(ogen) is resistant to inhibition by α2-antiplasmin. Another broad-spectrum proteinase inhibitor α2-macroglobulin can also inhibit plasmin. This nonserpin forms a noncovalent complex with plasmin; however, this inhibition takes place only when there is significant decrease in the local or systemic concentration of α2-antiplasmin [96].

3. Bacterial Plasminogen Activators

Several pathogenic bacteria, such as *Streptococcus* and *Staphylococcus*, also produce PAs that are either secreted or surface-bound proteins. Streptokinase (SK) and staphylokinase (SAK) are not enzymes in and of themselves but form 1 : 1 complexes with Plg and Pm, leading to changes in conformation and specificity of Plg. Crystal structures of SK and SAK reveal that they have similar structural fold, although sequence homology is relatively low [97, 98]. The mechanism of Plg activation by SK and SAK is similar but differs in some respects. While SK-Plg is enzymatically active, SAK-Plg is inactive and requires the conversion of Plg to Pm. Another important difference is that SAK primarily activates fibrin-bound Plg. This requirement has encouraged studies with SAK regarding its ability to function as an *in vivo* thrombolytic agent [99].

3.1. Activation of Human Plasminogen by Streptokinase. Invasive bacterial infections caused by *Streptococci* represent one of the most extensively studied models for interactions between pathogens and the Plg system [100]. *Streptococci* have the ability to invade nonphagocytic cells by breaking the host cellular and tissue barriers through interaction with host proteins that facilitate access into the vascular system [101, 102]. Interactions between the Plg system and streptokinase (SK) have been hypothesized to promote the bacterial invasion into tissues [103]. The SK, secreted by β-hemolytic group A, C, and G *streptococci*, is the prototypical bacterial plasminogen activator. It is a 440

amino acid protein containing α, β, and γ domains, with a 26 amino acid N-terminal signal peptide that is cleaved during secretion to yield a 414 amino acid mature protein [104]. Experiments have demonstrated that each individual domain can bind to Plg. However, they cannot activate Plg independently. Mutagenesis studies have shown that the α, β, and γ domains cooperatively induce the formation of an active site within the hPlg activator complex, providing a means for the substrate Plg to be recognized by the activator complex, mainly through interactions mediated by the SK α-domain [105, 106]. Interestingly, unlike other Plg activators which activate Plg by limited proteolysis, SK lacks hydrolytic activity. Thus, in order to activate Plg, SK has to bind to Plg and induce within it an active site through nonproteolytic mechanisms.

The activation of Plg by SK involves two major mechanistic steps: (1) formation of a PA complex and (2) the activation of substrate Plg. The crystal structure of SK from *S. pyogenes* in complex with the catalytic unit of Plg shows that the formation of the activator complex involves interactions between the carboxyl-terminal domain of SK and the catalytic domain of Plg [97]. In the SK-Plg complex, the active site of Plg is exposed and functions without hydrolysis of the Arg560-Val561 peptide bond [107]. Crystallographic studies have also determined the surface area of the respective SK domains available to interact with Plg and found that the interaction sites are located in the loops of α and the γ domains of the SK. In the second step of the activation, SK-Plg complexes become catalytic activators of the remaining Plg. The formation of the complex induces conformational changes, such that Plg is converted to Pm [108]. The Plg activation mechanism is also modulated by lysine-binding site- (LBS-) dependent interactions between SK and the kringle domains of Plg and Pm [109–111] which most likely involves K5 of Plg and β and/or β, γ domains of SK [105, 112].

Although the specific roles of the domains of SK that function in various ways in the activator complex have been revealed [113, 114], the basis for the species selectivity of SK in activation of mammalian Plg is still unclear [115, 116]. Human and nonhuman-derived SKs differ from each other and activate Plg in a species-specific manner. Early studies with rabbit Plg showed weak activation when incubated with SK produced by a strain of human origin. It was found that although a complex was formed between SK and rabbit Plg, SK was rapidly degraded to inactive forms, indicating that the complex was unstable [117]. Investigations with SKs isolated from *streptococcal* strains from equine or porcine origin have shown that although equine/porcine-derived SKs interact with hPlg, no activation was achieved. A similar situation was observed when either equine or porcine Plg was incubated with SK from a bacterial strain of human origin. Comparisons of amino acid sequences of different mammalian-derived SKs have shown that there is low sequence homology. These data indicated that the complex formed between SK and Plg could vary in primary structure and conformational properties, affecting Plg activation [118]. This remarkable species specificity is believed to originate from the species-specific interaction between SK and the preferred host's Plg. Using transgenic mice expressing human Plg it has been shown that a marked increase in the susceptibility of mice to group A *streptococcus* (GAS) was observed which is largely abrogated by deletion of the SK gene. These results demonstrate that SK is a key determinant for host specificity of *streptococcal* infection [119].

3.2. Activation of hPg by Staphylokinase. Staphylokinase (SAK) is a 136 amino acid protein produced by strains of *S. aureus*. SAK does not possess protease activity by itself. Instead, hPlg activation by SAK depends on the formation of a stoichiometric complex between SAK and Pm. The SAK-Pm is formed from SAK-hPlg in the presence of other PAs for example, tPA [120]. The SAK-Pm complex modifies SAK within the complex by cleavage of the Lys10-Lys11 peptide bond and subsequently converts SAK-Plg to SAK-Pm, which in turn converts free Plg to Pm. The kringle domains of Plg are not involved in the interaction with SAK, and there is evidence that Arg719 in Plg and Met26 in SAK are important for the binding [98, 121]. Moreover the NH_2-terminal region of SAK is important for active site formation in the Pm molecule in the binary complex [99, 121, 122].

Plg activation mediated by SAK differs from SK-mediated Plg activation in some major aspects. (i) The SAK-Plg complex is enzymatically inactive and requires conversion of Plg to plasmin. (ii) SAK requires fibrin as a cofactor. (iii) Active SAK-plasmin complex is efficiently inhibited by α_2-antiplasmin. However, binding of Plg to fibrin or bacterial cell surfaces protects against inactivation by α_2-antiplasmin and, more importantly, enhances the SAK-induced Plg activation [123]. (iv) Binding of α_2-antiplasmin to the SAK-plasmin complex releases SAK from the complex allowing it to interact with other plasmin(ogen) molecules [121, 124, 125]. SAK also binds much more efficiently to substrate-bound Plg than to soluble Plg [126]. Other than Plg activation, SAK can also induce immunogenic antibody responses and proliferation of SAK-specific T lymphocytes [127]. Furthermore, SAK can directly interact with the host innate immune system and inhibit the bactericidal effect of α-defensins, thereby reducing the rate of Plg activation. As a result, the functional activity of SAK promotes the bacterial infection process [128]. The observation that SAK primarily activates Plg bound to fibrin without causing systemic Plg activation has raised interest in the clinical use of SAK as a thrombolytic agent to dissolve the fibrin component of blood clots [99].

4. Bacterial Plasminogen Receptors

Plasminogen activator production is not universal amongst pathogenic bacteria but many bacteria express PlgRs on their surface [1, 129]. Bacterial PlgRs capture Plg on the bacterial surface which in turn enhances its activation to Pm by SK or host plasminogen activators uPA or tPA [130]. Consequently bacteria become proteolytic organisms using the host-derived system and thus facilitate bacterial penetration of endothelial cell layers and degradation of extracellular matrix

Table 1: Bacterial plasminogen receptors.

Receptor	Primary bacteria	Possible physiological function	Reference
Enolase/SEN	*Streptococcus*	Plg binding and uPA/tPA-mediated activation Fibrinolysis, subsequent extracellular matrix degradation, and transmigration. Involved in tumorigenesis, cancer proliferation, invasion, specific humoral and cellular immune response, myogenesis as well as hypoxic stress response	[10]
GAPDH/SDH/Plr	*Streptococcus*	Plg binding and activation by uPA/tPA. Adhesion to uPAR, fibronectin	[11, 12]
M proteins, PAM	*Streptococcus*	Major virulence factor, promote fibrinolysis, antiphagocytosis, vascular leakage and tissue injury, proinflammatory properties, adhesion to host cell and tissues, transmigration and deep tissue invasion, immunoglobulin binding, acute rheumatic fever	[13, 14]
OspA, OspC	*Borrelia*	Plg activation by host uPA, increased MMP expression, degradation of soluble and insoluble ECM components, endothelial monolayer penetration including blood-brain barrier, effective dissemination in host	[15–17]
HP-NAP	*Helicobacter pylori*	Fibrin clot stabilization, inhibition of fibrinolysis, possibly antiphagocytic, tissue factor synthesis and PAI2 stimulation	[18]
PgbA/PgbB	*Helicobacter pylori*	Lysine-dependent Plg binding, tPA-mediated Plg activation	[19]
DnaK	*Bifidobacterium animalis* subsp. *lactis*	Plg binding; upregulated in response to bile salts	[20]
Flagella	*Escherichia coli*	Plg binding and activation by host PAs	[21]
Fimbriae	*Escherichia coli Salmonella*	Fibronectin binding, adhesion to ECM components/BM, facilitate penetration	[22]

components. Additionally, immobilization of Pm protects it against the serine protease inhibitor α_2-antiplasmin [131]. Several bacterial PlgR molecules have been identified and characterized (Table 1). Most of the identified bacterial PlgRs have other important functions as well. In this paper we will focus only on the well-known and well-characterized bacterial PlgRs.

The best characterized PlgRs have been identified in group A and C *streptococci* from humans. These include α-enolase (SEN) [10] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; also known as SDH and Plr) [11, 12] as well as the *streptococcal* M-like protein (PAM) [13, 14]. Both GAPDH and enolase are glycolytic enzymes expressed on the bacterial surface [132, 133]. These are anchorless multifunctional proteins which, in addition to their housekeeping functions, are also involved in bacterial-induced fibrinolysis and inflammation through their ability to bind Plg.

Other than *streptococci*, GAPDH is also expressed on the surface of several Gram-positive bacteria including pneumococci as well as pathogenic *Escherichia coli* (*E. coli*) and binds to plasminogen, fibrinogen, and fibronectin binding proteins [133–135]. Enterohemorrhagic and enteropathogenic *E. coli* are reported to secret GAPDH which was found to bind human Plg and fibrinogen therefore suggesting a possible role in bacterial pathogenesis [135]. GAPDH (also known as *streptococcal* surface dehydrogenase—SDH) possesses lower affinity for Glu-Plg than Pm [133]. The C-terminal lysine residue of GAPDH appears to be essential for Pm binding as substitution of this residue with leucine abolishes binding. However, this replacement failed to show any effect on Plg binding indicating possible involvement of other *streptococcal* PlgRs [136]. It was found to be impossible to analyse the effect of GAPDH deletion on Pm binding since GAPDH appears to be essential for viability in GAS. However, analysis of swine pathogen *Streptococcus suis* mutant lacking cell surface GAPDH activity showed reduced Pm affinity. Lysine-dependent Plg binding by recombinant GAPDH isoform at physiological concentrations has been recorded for *Bacillus anthracis* [137]. Immunization of mice with this isoform offered significant protection against *Bacillus anthracis* infection. Taken together, these data establish the significance of GAPDH as a Plg receptor but also indicate that GAS probably expresses multiple PlgRs. In addition to its Plg binding activity, the GAPDH/SDH binds to the ectodomain D1 of uPAR/CD87 on Detroit human pharyngeal cells and mediates bacterial adherence to host cells [138]. SDH has also been shown to be involved in the regulation of phosphorylation of human pharyngeal cells which suggests a role for this enzyme in signal transduction and cell-to-cell signalling between *streptococci* and pharyngeal cells [139, 140]. GAPDH may also act as a virulence factor which could contribute to pathogenesis [135].

Like GAPDH, enolase is a glycolytic enzyme identified in archaebacteria to mammals. It is a highly conserved protein with similar overall fold and identical catalytic residues in

all organisms [141]. There are three isoforms of enolase—α, β, and γ which share high sequence identity. Of these, α-enolase is present in almost all tissues [142–144]. Enolase consists of a relatively small N-terminal domain and a rather large C-terminal domain. In some eubacteria, for example, *streptococci*, α-enolase is octameric [145], whereas in eukaryotes, enzymatically active enolase is present as an antiparallel dimer [146]. Enolase is a ubiquitous surface-associated protein although it is not clear how enolase is exported to the surface because it lacks the N-terminal signal peptide required for surface export as well as the membrane anchorage motif required for cell wall anchorage [10, 132, 145, 147]. Enolase is a multifunctional protein which, apart from being a key player in metabolism, is also involved in tumorigenesis, cancer proliferation, invasion, specific humoral and cellular immune response, and myogenesis, as well as hypoxic stress response (reviewed in [144, 148, 149]). Interestingly, surface-associated enolase serves another important function in both pro- and eukaryotes in that it acts as a PlgR by binding to and activating host Plg [132, 150–155] on cell surfaces and thereby facilitating fibrinolysis and subsequent extracellular matrix remodelling. This may in turn assist in bacterial invasion of host cells. Binding leads to activation of Plg to Pm by the action of tPA or uPA [156, 157]. Enolase binds to kringles of Plg in a lysine-dependent manner. C-terminal lysine residues are critical for binding to Plg as the lysine analog ε-aminocaproic acid significantly inhibits binding as has been shown in *Borrelia* and human pharyngeal cells [158, 159]. However, ionic interaction does not affect this binding [158]. Lysine residues at positions 420 and 427 of enolase were found crucial in Plg-binding activity in *Aeromonas hydrophila* SSU [160]. It was also found that immunization of mice with purified recombinant enolase significantly protected the animals against a lethal challenge dose of wild type (WT) *A. hydrophila* suggesting that enolase could potentially be important for the viability of the pathogen [160]. Similar to GAPDH, α-enolase shows greater affinity for Lys-Plg than to Glu-Plg [161]. Lys-Plg-coated GAS resulted in increased adherence of GAS to human pharyngeal cells (Detroit 562) that have α-enolase expressed on their surface [159]. Additionally, in pneumococcal enolase, a nine-residue motif [FYDKERKVY] has been found to be the key cofactor for Plg binding and degradation of ECM proteins as well as important for dissolution of fibrin or laminin and transmigration of pneumococci through fibrin matrices [162]. Using an intranasal mouse infection model it has been shown that the functional inactivation of the nine-residue motif significantly impairs virulence of *streptococci* [163]. This motif has been found to be pivotal in other pathogens as well [144, 160]. However, the full conservation of the motif does not seem to be necessary for Plg activation [164]. Interestingly, pretreatment of mice with recombinant enolase from *Streptococcus sobrinus* suppressed the primary immune response against T-cell dependent antigens and also induced an early production of the anti-inflammatory cytokine, interleukin-10, thereby suggesting that surface enolase might act as an immunosuppressive agent [165]. Because of its location and possible function, enolase therefore has the potential of being a therapeutic target [149, 166].

Perhaps the best characterized bacterial PlgRs are M proteins secreted by group A *streptococcus* (GAS). GAS is a highly specific human pathogen. Its efficient colonization and dissemination in the host lead to a broad spectrum of diseases that range from simple and uncomplicated pharyngitis, tonsillitis, and skin infections, for example, impetigo, to life-threatening invasive illnesses including pneumonia, bacteremia, necrotizing fasciitis, *streptococcal* toxic shock syndrome, as well as nonsuppurative complications like acute rheumatic fever, and glomerulonephritis [167]. GAS produces a variety of surface-bound and secreted virulence factors which are known to contribute to the severity of their infections [168]. Among these, M- and M-like protein PAM (plasminogen binding M-like protein), which are anchored to the cell wall, play key roles in bacterial resistance to phagocytosis, adherence, invasion, and microcolony formation in tonsillar tissue (reviewed in [168]). M and M-like proteins function through various mechanisms for effective dispersion of the bacteria in the host. M proteins enable GAS to resist host immunity and invade the host by binding to IgG and IgA, as well as other proteins of the innate immune system. Apart from its role as antiphagocytic, M and M-like proteins also interact with and stimulate activation of the host fibrinolytic system [119]. M and M-like proteins of GAS are able to bind to host Plg directly [13, 35] or indirectly via fibrinogen (Fg) and/or fibrin (Fn) [169, 170] and serve to enhance the activation of Plg to Pm. M proteins have been studied extensively since their discovery in 1928 and are now amongst the best studied virulence factors of pathogenic bacteria [167, 171]. M proteins exhibit extensive sequence variations between strains accounting for over 130 distinct serotypes, each one exhibiting unique antigenic properties [172]. This enormous diversity of GAS serotypes is a major obstacle in the development of vaccines against GAS.

M proteins adopt a dimeric α-helical coiled-coil structure [173] tethered to the membrane at their C-terminal end. The hypervariable N-terminal region, which extends into the external environment, varies extensively in sequence between different bacterial strains and gives rise to the so-called antigenic variation in M proteins. M proteins consist of several domains of distinct functionality. The first 40 residues, which form the signal sequence, are absent in the mature protein. Mature PAM proteins begin with a variable P domain that contains two 13 amino acid repeats termed a1 and a2. This is followed by three C repeats and a D domain all of which are highly conserved across the M-protein family. These sequences bind a variety of human plasma proteins including members of the complement system. The highly conserved Pro/Gly domain is inserted in the GAS cell wall and anchors PAM on the bacterial surface. PAM binds to the kringle 2 (K2) domain of hPlg via its a1a2 domain [13, 174] whereas M proteins on other GAS strains bind to Fg through B repeats, which in turn interacts with Plg via K1, K4, and/or K5 domains of Plg [170, 175]. Binding of PAM to hK2/hPlg is mediated mostly by R72, H73, E75, R85, and H86 residues in the a1/a2 repeat of native PAM. Substitution of the central lysine in the a1 repeat sequence DAELQRLKNERHE reduced

Plg binding by 80% [14]. The role of the a1 and a2 regions in Plg binding was further demonstrated by expressing the a1 and the combined a1a2 regions as chimera to Arp4, an M-like protein which lacks Plg binding. Not only did the recombinant chimeric Arp/PAM proteins bind to Plg and Pm but also the expression of these fusion proteins conferred Plg binding on the *streptococcal* host strains lacking the Plg-binding ability [171]. In the presence of Plg, the a1-Arp4 fusion in an SK-producing strain resulted in surface-associated plasmin activity, whereas a host strain with an inactivated SK gene required exogenous SK for Plg activation [142].

PAM is encoded by the emm53 gene, which is associated with the GAS strains that cause skin infections in humans [176, 177]. Inactivation of emm53 leads to a loss of Plg binding *in vitro* and attenuated infection in an experimental model of impetigo as well as in transgenic mice expressing human Plg. The surface-bound Plg can be activated by SK or host PAs. In this way, PAM concentrates Plg on the surface of the bacteria and greatly augments the SK-mediated conversion of hPlg to hPm. PAM appears to work synergistically with SK to yield bacteria-bound Pm *in vivo*.

5. Mechanisms of Pathogen Invasion Using Host and Bacterial Proteins

5.1. Interaction of Bacteria with the Fibrinolytic Cascade via Plasminogen Receptors. The connection between microbial infection and fibrinolytic system has been known since 1933 when haemolytic *streptococci* from human infection samples were found to possess fibrinolytic activity [178]. Speculation was rife when it was reported that injection of *streptococcal* cell wall products triggered activation of the coagulation pathway in rats [179]. The binding of bacterial Plg receptors to Fg has been extensively studied mostly in GAS [180]. Plasminogen deficient ($Plg^{-/-}$) mice have been utilized in several pathogen challenge models. Binding of host Fg to M proteins of GAS enhances SK function in plasma. Using a mouse skin infection model it has been shown that GAS isolates with a surface-bound SK-human Plg-human Fg complex are invasive when injected into the skin of wild-type (WT) mice but not $Plg^{-/-}$ mice [24]. This also demonstrates that PlgR interaction with host Plg facilitates bacterial invasion. This supports the *in vitro* observation that GAS incubated with human plasma acquires Pm activity on its surface [181]. In subcutaneously infected mice, coadministration of human Plg considerably increased the susceptibility to GAS infection which was largely abolished by inactivation of the SK gene [103]. Similarly, inactivation of SK gene leads to a loss of virulence in a human-skin-mouse model for impetigo [182]. In an epidemiological study, GAS strains expressing M proteins isolated from invasive infectious cases were found to bind more Plg than strains isolated from noninvasive infectious cases in the presence of Fg and SK [183]. These results demonstrate the importance of bacterial Plg receptors in host pathogen interaction and indicate that SK and PlgRs probably share a synergistic action. A transgenic line of mice that express only human Plg has been generated to study the role of the host fibrinolytic system in GAS infection [119]. These mice showed increased mortality and increased bacterial dissemination, when subcutaneously infected with GAS. The presence of M protein was found to be required for full scale virulence. In this infection model it was also demonstrated that decreasing Fg levels facilitated bacterial spread thereby suggesting that in the absence of Pm activity, fibrin clot formation can be an effective host-defense mechanism. Fibrin-mediated protection to infection-stimulated hemorrhage has also been documented in mice infected with *Toxoplasma gondii* [184]. In an infection study using $Plg^{-/-}$ mice and mice deficient in Plg activators, it was observed that during infection with *Mycobacterium avium* the liver granulomas in $Plg^{-/-}$ mice had enhanced fibrin and fibronectin deposition, as well as increased neutrophil infiltration suggesting that Pm plays a role in the turnover of extracellular matrix proteins within granulomas, likely limiting the fibrotic response in these lesions [185].

The binding of Fg to M protein may also play a role in modulating bacterial-phagocyte interaction. Fg-M protein complex inhibits complement deposition on the bacterial surface thereby protecting GAS from phagocytosis [169]. M protein-Fg complex can bind to $\beta 2$ integrins, thus activating neutrophils and consequently inducing vascular leakage that could cause extensive pulmonary damage [186]. This further establishes the importance of Fg in pathophysiology of *streptococcal* infection. M protein-Fg interaction and subsequent activation of coagulation can have a profound influence on clot modulation of human plasma [187]. M proteins can target both intrinsic and extrinsic pathways of coagulation which are critical for the induction of fibrin clot formation [179, 188–190]. M proteins can also trigger platelet aggregation *in vitro* and thereby play a role in primary hemostasis. Fg was later found to be required for *streptococcal*-induced platelet aggregation [191]. Severe *streptococcal* infection is associated with M protein-induced platelet activation and thrombus formation [192]. M protein is a multipotent and powerful inducer of inflammation. It can also interact with Toll-like receptor 2 on human blood monocytes [193], resulting in expression of cytokines and tumor necrosis factor-α [194]. These proinflammatory roles of M proteins may explain why aggregates of M protein and platelets are often found at the site of infection in patients with toxic shock syndrome. GAS-associated fibrinolysis is potentially necessary for facilitating bacterial access to the vasculature [119].

5.2. Plasminogen-Mediated Bacterial Invasion. Plasminogen activation is also important for the tissue barrier degradation that allows cell migration. Through activation of metalloproteases, Pm-coated bacteria can break down extracellular matrix and basement membranes either directly or indirectly thereby facilitating bacterial spread [195]. In *Salmonella enterica* it has been demonstrated that Pm activity associated with the bacterial surface is able to degrade laminin as well as mammalian ECM and can potentiate *in vitro* bacterial penetration through a reconstituted BM [196]. Plg binding in *Borrelia* resulted in enhanced penetration of endothelial

cell monolayers [197]. In *S. aureus*, α-enolase has been suggested to play a critical role in bacterial pathogenesis by allowing its adherence to laminin-containing extracellular matrix [198]. In an infection study of bovine mammary epithelial cells by *S. aureus*, it has been reported that addition of Plg to the medium enhanced dissemination and invasiveness of bacteria [199]. Pneumococci also bind to and activate Plg via enolase which mediates transmigration across endothelial and epithelial monolayers by intercellular junction cleavage [200, 201]. α-enolase also showed enhanced monocyte migration through epithelial monolayers and promoted matrix degradation *in vitro*. These effects were abrogated by antibodies directed against the Plg binding site of enolase. Enolase also promotes Plg-mediated recruitment of monocytes to the acutely inflamed lung. These data suggest an important mechanism of inflammatory cell invasion mediated by increased cell-surface expression of enolase [202]. *Bacillus anthracis*, the causative agent of anthrax, is known to circumvent the humoral and innate immune defense of the host to initiate a productive infection. Enolase has been found to be at the helm of Plg binding which makes the Plg-bound spores capable of exhibiting antiopsonic properties by cleaving C3b molecules, *in vitro*, resulting in a decrease in macrophage phagocytosis [203]. This study showed that recruitment of Plg by PlgR can assist in evading host innate immunity also by the enhancement of anticomplement and antiopsonization properties of the pathogen. It is noteworthy that fibrillar surface appendages like fimbriae, flagella that have a morphological similarity to fibrin, form a major class of PlgR molecules in gram-negative bacteria, for example, *E. coli*. Pathogenic *E. coli* and *S. typhimurium* can bind to Plg by their thin aggregative fimbriae (curli) and/or flagellar filaments resulting in Plg activation by tPA [21, 22]. These organelles have been found to adhere to ECM or BM proteins, indicating that these bacterial structures are possibly involved in the invasion of host tissues as well as promotion of bacterial colonization (reviewed in [204, 205]). *E. coli* strains isolated from patients with ulcerative colitis showed higher affinity to various solubilized ECM proteins compared to those from healthy subjects [206]. This may indicate the existence of a positive correlation between PlgR activity and pathogenicity.

5.3. Plasminogen-Dependent Tissue Invasion by Borrelia . The importance of Pm acquisition for invasion and dissemination has been demonstrated for the vector borne spirochetes *Borrelia burgdorferi*. These bacteria are transmitted by ticks to human skin where they invade the outer skin layers and disseminate in the blood to cause Lyme disease [207]. They also migrate to secondary infection sites, such as the joints, the heart, and the central nervous system and, over time, Lyme disease can evolve into erosive arthritis and the destruction of ECM in the joints. *Borrelia* secretes two Plg-binding surface proteins—OspA and OspC [15–17] which are differentially expressed in host and ticks. In unfed ticks, OspA is highly expressed on the surface of spirochetes. After infection in mammals, OspA expression is downregulated and OspC is constitutively produced [208]. *Borrelia*-bound Pm promotes degradation of soluble and insoluble components of ECM [209] and penetrates endothelial monolayers [197] including the blood-brain barrier [210]. A similar observation has been made recently with *Cryptococcus* where blood-brain barrier invasion by the pathogen is enhanced by functional interaction with Pm [7]. Studies in $Plg^{-/-}$ mice demonstrated that Plg is required for effective dissemination of *Borrelia* in ticks and for enhancement of spirochetemia in mice [211]. *Borrelia* do not produce collagenases. However, increased expression of MMPs including MMP-1, MMP-3, and MMP-9 were observed in cell culture infections with *Borrelia* [212–216]. It is noteworthy that surface-bound Pm in *S. aureus* activates the proteolytically active interstitial collagenase MMP-1 in a similar way [217]. Using $Plg^{-/-}$ mice, it has been demonstrated that during relapsing fever from Borrelia infection, a lack of host Plg resulted in decreased spirochetal burden in the hearts, brains, and kidneys of these mice compared to WT mice. Neurological symptoms were also less common in $Plg^{-/-}$ mice [218, 219]. Interestingly, there is no endogenous PA in *Borrelia*; instead, the bacteria use host uPA to activate surface-bound Plg. Results from these studies suggest that receptor-bound Plg promotes tissue invasion in *Borrelia*.

5.4. Interaction of Helicobacter pylori with the Plasminogen System. In vitro binding of Plg has also been reported for *Helicobacter pylori* isolates which is associated with severe gastrointestinal disorders including chronic gastritis, peptic ulcers, and stomach cancer [220, 221]. Binding is sensitive to lysine analogs, and bound Plg is activated by tPA [222]. *H. pylori* produces a dodecameric neutrophil activating protein (HP-NAP) that can potentially inhibit fibrinolytic activities [18]. This study showed that HP-NAP efficiently stimulates human monocytes to synthesize tissue factor (TF) and plasminogen activator inhibitor-2 (PAI-2) without altering uPA production. Clotting assays established that tissue factor (TF) is functionally active thus triggering blood clotting and promoting fibrin deposition. As a result, the coagulation-fibrinolysis balance is shifted towards fibrin formation, prothrombotic events, and fibrin clot stabilization. This is perhaps beneficial for chronic gastritis and tissue disruption which requires tissue healing by means of degradation and removal of fibrin deposits and tissue debris. Fibrin deposition might in turn protect the bacteria against phagocytosis. In gastric cancer cells and in gastric biopsies of *H. pylori* positive patients, an increased level of PAI-2 has been demonstrated [223]. These data were also supported by the observation that in *H. pylori*-infected patients an increased level of PAI-2 and TF expression in monocytes was detected suggesting the involvement of pathogen in the regulation of the fibrinolytic balance and, hence, disease development [224]. Apart from HP-NAP, two more genes (pgbA and pgbB) encoding surface-exposed proteins with Plg receptor activity have been identified and characterized from *H. pylori* [19]. Both bind to Plg specifically in a lysine-dependent manner and enhance tPA-mediated Plg activation. In both cases, Plg binding has been

shown to be located in the conserved C-terminal region which contains two lysine residues.

5.5. Other Bacterial Pathogens and the Plasminogen System. In *Porphyromonas gingivalis* and the plague bacterium *Yersinia pestis*, plasmin inhibitors α_2AP and α_2-macroglobulin (α_2M) are inhibited [225, 226]. Since both of these pathogens also activate Plg, specific inactivation of antiproteases will therefore lead to uncontrolled Pm activity resulting in ECM and BM degradation [195]. Suppressing host antiproteases is therefore another effective mechanism undertaken by bacteria to promote proteolysis and invasion [227]. *Y. pestis*-derived plasminogen activator (Pla) is a surface protease responsible for the invasive character of plague. This pathogen does not express any Plg or Pm receptors; instead Pla acts as a highly efficient Plg activator [226] which proteolytically cleaves Plg similar to host uPA and tPA [3]. It is also an adhesin with affinities for ECM, laminin, and BM [228]. $Plg^{-/-}$ mice were found to be more resistant to plague infection than normal mice therefore validating the involvement of Pla in Plg activation [229].

Together, these observations clearly demonstrate that host Plg plays a critical role in pathogenesis of a broad range of invasive pathogens.

6. Concluding Remarks

The experimental evidence discussed here clearly demonstrates that a vast number of bacterial pathogens express PlgRs and interact with the host Plg system which in turn results in enhanced bacterial virulence. It is also clear that pathogenic bacteria can, and most often do, use the Plg system for migration across tissue barriers. Most of the identified and characterized bacterial PlgRs are multifunctional proteins involved in other important functions as well, such as adhesion, enzymatic activity, movement, interaction with immune system, myogenesis, and antiphagocytosis. It is also noteworthy to mention that a single bacterial species can express multiple PlgRs most likely due to the affinity of Plg for carboxy-terminal and internal Lys residues. Recent reports show that nonpathogenic bacteria can also express PlgRs and bind Plg via internal lysines [20, 230, 231]. Probiotic and commensal lactobacilli express an enolase, which, similar to staphylococcal enolases, interacts effectively with Plg and laminin. [232]. However, the significance of these expressions or the possible effects on colonization are not yet known.

During the last few decades various studies have suggested that there may be a correlation between the pathogenic invasion and the host haemostatic mechanisms, mainly fibrinolysis. Experimental results discussed here also demonstrate the complicated relationships of fibrinogen with invading bacteria and suggest a possible manipulation of the host fibrinolytic cascade and the receptor molecules of the pathogens to evade the host defense system. Not only is the Plg system important in controlling fibrin-containing clot degradation under physiological and pathophysiological conditions but this system can also be hijacked by bacterial pathogens to create exuberant proteolysis resulting in extracellular matrix and fibrin barrier degradation, subsequent tissue damage, and enhanced bacterial migration and invasion into the surrounding host tissue and vasculature. These properties therefore highlight the fibrinolytic system as a novel therapeutic target for controlling pathogenic invasion and dissemination.

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