Invasive bacterial infections are major clinical problems. The emergence and spread of antibiotic resistance in bacteria is becoming a growing health threat. The key to finding novel treatments for human bacterial infections lies in understanding the interactions between the host and pathogen. During bacterial infection, the host responds to invading microbes with a number of different defense mechanisms. Some bacteria can, however, circumvent the host defense. In severe infections, such as sepsis and septic shock, the coagulation and fibrinolytic systems are compromised. The coagulant/anti-coagulant balance is disrupted, causing severe thrombosis or bleeding (2, 42, 54, 56, 57). Hemostatic factors hold the potential as novel therapeutic targets for treatment of bacterial infection-related diseases. However, we are just beginning to understand the function of hemostatic system in host/pathogen interaction.

The infectious process starts with the first contact between the bacteria and the human host. Different bacterial species gain access to the human body through different sites, such as the skin, nasopharynx, lungs, gastrointestinal, or urogenital tract. Bacterial invasion is generally mediated by bacterial surface and secreted products that can negate host innate and acquired defense systems. Both the ability to produce invasive molecules and to elude host defense are critical for causing systemic diseases (10, 16).

The hemostatic system plays an important role in systemic infection. An interesting example of the interaction between host coagulation system with pathogens is the horseshoe crab, which uses endotoxin to trigger a clotting response that presumably walls off the bacteria, providing an initial defense against invasion (39, 41, 70). The clotting system of horseshoe crab consists of three serine proteases and one clottable protein that are functionally similar to mammalian fibrinogen and share some sequence homology with primate fibrinopeptide B (40, 71). This observation establishes the clotting system as one of the primitive parts of the host defense against bacterial infection. This coagulation response to bacterial infections also appears to be preserved in mammals, in which infections trigger tissue factor expression on the surface of monocytes, which in turn initiates the coagulation cascade (73, 85).

The Coagulation System

The primary function of the coagulation system is to stop bleeding on an injury until repair occurs. Through the activation of a cascade of plasma proteins, blood clots are formed at the site of injury or blood flow disturbance through fibrin deposition and platelet congregation (FIGURE 1) (24). In the event of injury to blood vessel wall, tissue factor (TF) normally sequestered in the subendothelial layer is exposed. TF binds and activates circulating factor VII (FVII). The complex of TF and activated FVII (FVIIa) will then activate factor X (FX) and factor IX (FIX). Activated FXa will form prothrombinase complex with activated factor V (FV). The prothrombinase complex (FVaFXa) then cleaves prothrombin to thrombin, which then cleaves fibrinogen to form fibrin. FIX activation also amplifies the clotting reaction through interaction with activated factor VIII, which accelerates FX activation.

Overactive coagulation will cause widespread thrombosis. Therefore, it has to be kept in check by anticoagulation systems. This is achieved through anticoagulation and fibrinolytic systems. Together, coagulation, anticoagulation, and fibrinolysis maintain a delicate physiological balance (FIGURE 1).

The main function of the anticoagulation system is to prevent or slow the propagation of clots. The major anticoagulants include antithrombin (AT), tissue pathway inhibitor (TFPI), and activated protein C (APC). AT is produced by the liver and inhibits several coagulation factors such as thrombin, FVIIia, FIXa, and FXa (80, 84). TFPI is a serine protease that inhibits FXa. In the presence of FXa, TFPI also inhibits the TF-FVIIa complex (12, 13, 79). Protein C is an inactive plasma serine protease. When thrombin is produced, it can bind to thrombomodulin present on the vascular endothelial surfaces. The thrombin/thrombomodulin complex can then cleave protein C into APC. APC generation is enhanced by the endothelial cell protein C receptor (EPCR) on the endothelial surface. APC, with cofactor protein S, can cleave and inactivate FVa and FVIIia to negatively regulate coagulation (1, 23, 29).

The fibrinolytic system functions to break down existing fibrin clots. The major protease of fibrinolytic
The initial observation of interactions between microbes and the fibrinolytic system was made in the 1930s. Tillett and Garner (93) observed that streptococci from human infection samples possessed fibrinolytic ability. On the other hand, isolates from veterinary infections did not produce lytic activity for human fibrin (93). Several other studies also suggested that the fibrinolytic activity of \( \alpha \)-hemolytic streptococci was species specific and dependent on the source of the pathogenic isolate. Streptokinase (SK) was identified as the streptococcal plasminogen activator (63, 64, 72, 86). SK has been proved to be useful clinically in dissolving blood clots that cause heart attacks (60, 68). The crystal structure of the catalytic domain of human plasmin complexed with SK has been solved (97). SK can convert plasminogen into an active serine protease without proteolysis. Formation of the SK/plasminogen complex can induce conformational change in the activation domain of plasminogen. This complex can hydrolytically activate other circulating plasminogen molecules. Furthermore, this complex is resistant to host plasmin inhibitors such as \( \alpha \)-antiplasmin. Streptococci are proposed to gain fibrinolytic activity through two independent pathways (10). One pathway involves the direct binding of plasmin to the bacteria surface by high-affinity molecules, such as PLG-binding group A streptococcal M-like protein (PAM) (102). In another pathway, streptococci form a SK/plasminogen complex with fibrinogen as a cofactor (62, 95, 96).

The interaction of SK with host plasminogen is highly species specific. SK generated by streptococcal isolated from different host species can only activate plasminogen from that host (63, 64, 86). It was demonstrated that human plasminogen can only be activated by SK generated by human pathogenic streptococcal stains. Mice are generally very resistant to infection by human pathogenic group A streptococcal (GAS). This remarkable species specificity of streptococcal infection has been proposed to result from the species-specific interaction between SK secreted by GAS and the preferred host’s plasminogen. Transgenic mice expressing the human plasminogen protein have been generated to study the role of the fibrinolytic system in GAS infection. A marked increase of susceptibility to GAS was observed in these transgenic mice in a subcutaneous infectious model. Furthermore, the increased susceptibility of the transgenic mice to GAS infection is largely abrogated by deletion of the SK gene. These results demonstrate that SK is a key determinant for host specificity of streptococcal infection (91).

Similar to \textit{Streptococcus}, \textit{Staphylococcus aureus} makes staphylokinase (SAK), which is also a plasminogen activator. Unlike SK, SAK has to activate plasminogen through proteolysis. The SAK/plasmin complex is sensitive to inhibition by \( \alpha \)-antiplasmin inhibitor, whereas the SK/plasmin complex is not (30). However, in vivo data on the importance of SAK in...
**Staphylococcus** pathogenecity is lacking. Based on the studies on SK, it is likely that SAK also plays a critical role in the virulence of *Staphylococcus*. Plasminogen activator has also been implicated in the pathogenesis of plague by *Yersinia pestis* (90). It was shown that *Y. pestis* required a 9.5-Kb plasmid for virulence when injected subcutaneously in mice. The virulence plasmid encoded a protease, the product of pla gene, which demonstrated plasminogen activator activity and weak coagulase activity. If the plasmid was inactivated, the lethality of the bacteria was decreased by a million fold (65, 66, 88, 89, 90).

A number of plasminogen receptors (PlgR) have been identified in group A and C streptococci isolated from humans (Table 1). These PlgRs include glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) (8, 101) and α-enolase (SEN) (74), as well as the streptococcal M-like protein (PAM). PAM binds plasminogen with high affinity and without the assistance of fibrinogen (3, 5, 81, 83). The surface-bound plasminogen can be activated by SK or host plasminogen activator. In this way, PAM concentrates plasmin on the surface of the bacteria. The role PAM plays in the streptococcal interaction with host plasminogen was investigated with mice with or without circulating human plasminogen (91). Human plasminogen markedly increased mouse susceptibility to PAM+ strain infection, suggesting that concentrating host plasminogen/plasmin on the surface of bacteria enhanced bacterial invasion. Furthermore, mice with human plasminogen exhibited higher susceptibility to PAM+ strain than to PAM- strain, demonstrating that interaction of PAM with host plasminogen facilitated bacteria invasion. Epidemiological studies also demonstrated the importance of streptokinase and plasminogen in host/GAS interaction. It was shown that, among PAM+ GAS strains, strains isolated from invasive infectious cases bound more plasminogen than strains isolated from noninvasive infectious cases in the presence of fibrinogen and streptokinase (67), supporting the studies in the murine model. Furthermore, streptokinase gene expression was significantly higher in the

<table>
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<tr>
<th>Bacteria</th>
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<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>SK</td>
<td>PLG</td>
<td>Activation</td>
<td>Increase virulence</td>
<td>59, 91</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td><em>Yersinia pestis</em></td>
<td>Pla</td>
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<td>Activation</td>
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<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>GAPDH</td>
<td>PLG</td>
<td>Binding</td>
<td>Adhesion to uPAR/antiphagocysis</td>
<td>8, 101</td>
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<td><em>Streptococcus pyogenes</em></td>
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<td>PLG</td>
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<tr>
<td><em>Streptococcus pyogenes</em></td>
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<td>3, 5, 81, 83, 91</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>Flagella</td>
<td>PLG</td>
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<td>Enhancement of formation of plasmin by host plasminogen activator</td>
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<tr>
<td><em>Escherichia coli</em>, <em>Salmonella typhimurium</em></td>
<td>Fimbriae</td>
<td>PLG</td>
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<td>49, 75</td>
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<td><em>Borrelia burgdorferi</em></td>
<td>Outer cell surface lipoprotein A</td>
<td>PLG</td>
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</table>
Collectively, these observations demonstrate that host plasminogen plays a critical role in pathogenesis of a broad range of invasive pathogens.

### Interaction of Fibrinogen with Pathogens

Fibrinogen plays multiple roles in the bacterial/host interaction. At the site of a local microbial infection, a number of secreted bacterial products, such as endotoxin, in conjunction with the cascade of cytokines released by host inflammatory cells induces a vigorous response in the surrounding host vasculature, including high-level expression of tissue factor, PAI-1, and a variety of other prothrombotic factors, that triggers the coagulation cascade to form thromboses. The resulting local vascular thrombosis serves to wall off the site of infection and limit pathogen invasion and spread. As discussed above, bacterial plasminogen activators (PA) such as SK appear to overcome this thrombotic host defense, including endogenous PAI-1 and α₂-antiplasmin, by activating plasmin to dissolve fibrin clots and clear the surrounding vasculature to facilitate bacterial spread (FIGURE 2). Therefore, fibrin clots should play an important role in stopping bacteria spread.

To test the role of fibrin in GAS pathogenesis, mice were treated with ancrod, a snake venom that can degrade plasma fibrinogen. Ancrod-treated mice exhibited marked increased susceptibility to streptococcal infection localized to the right flank subcutaneous tissue, suggesting decreasing fibrinogen level facilitated bacterial spread (91). Further test was per-

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**FIGURE 2. Model of bacteria invasion using host fibrinolytic system**

Bacterial plasminogen activators such as streptokinase (SK) facilitates bacterial invasion. **A:** at the site of bacterial infection (GAS), a vigorous inflammatory reaction is induced to release a cascade of cytokines, which will activate local coagulation system by inducing the expression of coagulation factors such as tissue factor (TF) and PAI-1, resulting in formation of local thrombosis. Host fibrinolytic system (PLG) is insufficient to dissolve the thrombosis due to the inhibition of PAI-1. **B:** invasive bacteria such as GAS produce plasminogen activators such as SK to activate host plasminogen (PLG) and form a complex of SK/plasminogen (SK/PLG). Since the SK/plasminogen complex is resistant to host fibrinolytic inhibitors, bacteria are able to dissolve local thrombosis to spread systemically.

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severe invasive GAS isolates than in the noninvasive isolates in Japan (38).

Many invasive bacteria recruit plasmin to their surface, making plasmin insensitive to α₂-antiplasmin inhibition. Plasmin-coated bacteria can degrade complement and immunoglobulins (90). Furthermore, these plasmin-coated bacteria can break down extracellular matrix and basement membranes either directly or indirectly through activation of metalloproteases, facilitating the bacterial spread (50). A marked increase in systemic bacteria spread was observed in mice with circulating human plasminogen infected locally with several GAS strains, suggesting that activation of host plasminogen facilitated bacteria invasion (91).

A large number of bacteria, such as *Borrelia burgdorferi* (35), *Escherichia coli* (49, 53, 75), *Salmonella typhimurium* (48, 49, 52), *Neisseria meningitides* (94), and *Haemophilus influenzae* (87), bind plasminogen yet rely on the plasminogen activators of the host to generate enzymatically active plasmin (Table 1). Plasminogen binding to bacteria can almost be considered a universal event (10, 16, 51, 95, 96). *B. burgdorferi*, which causes lyme disease, binds plasminogen and utilizes host uPA to convert plasminogen into plasmin to degrade extracellular matrix components (18, 34). Plasminogen-bound bacteria has been shown to be able to penetrate endothelial cell monolayers (19). Plasminogen was required for efficient dissemination of *B. burgdorferi* within the tick and for enhancement of spirochetemia in mice but was not critical for transmission and infection according to studies on plasminogen knockout mice (17).
formed by introducing bacteria directly into circulation. In this infection model, SK activation of human plasminogen would not play a prominent role in facilitating bacteria spread in mice. The difference in susceptibility to GAS infection between mice with and without human plasminogen was negated in this systemic infection model, supporting the role of fibrin clots in host defense against systemic invasion (91). Fibrin was also shown to be able to protect mice from infection-stimulated hemorrhage caused by Toxoplasma gondii (44).

In addition to its hemostatic function, fibrinogen also plays important roles in the inflammatory response. Fibrinogen has been shown to alter leukocyte functions, including cell adhesion, migration, and cytokine expression. Leukocyte integrin receptor α5β1/Mac-1 binds immobilized fibrinogen and regulates leukocyte activities. Mice with genetically engineered fibrinogen lacking the α5β1/Mac-1 binding motif showed severely compromised inflammatory response to bacterial infection. The leukocyte clearance of infected Staphylococcus aureus was significantly decreased in the peritoneal cavity (31). Fibrinogen may also play a role in modulating bacterial-phagocyte interaction. All pathogenic GAS isolates express cell-wall anchor M proteins. These M proteins are members of the M-protein family and can bind fibrinogen. It has been shown that GAS binds fibrinogen to resist phagocytosis. The mechanism whereby fibrinogen binding can contribute to phagocytosis resistance remains to be elucidated. Fibrinogen could either mask the conserved targets for antibodies in the streptococcal wall or compete with streptococcal associated C3bi for the binding to streptococcal wall or compete with streptococcal associated C3bi for the binding to streptococcal asso-

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These observations demonstrate the complicated relationships of fibrinogen with invading bacteria. As one of the most abundant plasma proteins, fibrinogen serves as the interface of coagulation and inflammation, at the same time, becoming one of the major players in bacteria/host interaction.

Genetic Variations in Coagulation and Host Response to Infections

Vascular inflammation and thrombosis are interrelated (27, 56, 57, 58). In the event of bacterial infection, the inflammatory response can induce TF expression in monocytes via nuclear factor κB (NF-κB) activation, thus initiating coagulation (9). On the other hand, coagulation can result in vascular cell activation to recruit leukocytes. Thrombin generation can also induce expression of cytokines from endothelial cells and mononuclear cells (43). A number of animal studies demonstrated that decreasing thrombosis improved survival when challenged with bacterial infections and endotoxin (21, 55, 77, 99). Among various animal models and clinical trials in sepsis patients, improved survival was noted in the APC-treated groups (6). The mechanism of APC’s function in sepsis treatment is still unclear. It is shown that APC can block the production of TNF, both in circulation and in tissue (69, 103). APC can also block NF-κB in monocytes and endothelial cells to inhibit the induction of inflammatory cytokines and adhesion molecules (46, 100). APC may also downregulate genes that are upregulated in inflammation and upregulate anti-apoptotic genes (36, 45). It appears that coagulation and inflammation are intertwined in a complicated network, and APC pathway is at the crossroads.

A number of host hemostatic proteins have been implicated in deciding the susceptibility of host to bacteria infection. Individuals vary considerably in their susceptibilities to infections and in their abilities to recover from infections. Like many complex trait genetic diseases, the molecular mechanisms underlying susceptibility to bacterial infections involve multiple genes in the immune, inflammatory, and coagulation systems. The differences in host susceptibility can be partially explained by polymorphisms of these genes. Recent studies have demonstrated that a common genetics polymorphism in coagulation factors, FV Leiden, improved the survival rates in both sepsis patients and mice challenged with endotoxin LPS (47). Of note, LPS is a pervasive toxin produced by the gram-positive bacteria. LPS can promote cytokine production in the mice, which in part mimic the septic shock syndrome observed in human sepsis cases (7). This observation provided further evidence of the importance of hemostatic system on host susceptibility to bacteria infection. FV is a central regulatory protein in the blood coagulation cascade. It is located at the crossroads of the procoagulant and anticoagulant pathways. It serves as a critical cofactor for FXa to form the prothrombinase complex, which cleaves prothrombin to active thrombin, whereas FVa is also the proteolytic target for APC. APC cleaves at position 504 and 305 of FV protein. Three independent polymorphisms in FV gene have been described: FV Cambridge
An intriguing question to study host/pathogen interactions remains: Are there polymorphisms in host hemostatic factors under positive selection due to bacterial infections? Bacterial infection is believed to be one of the most important selective forces in evolution. A classic example of balance-positive selection is the resistance against malaria conferred by sickle cell anemia mutation. FV Leiden mutation may also confer selective advantage against infections, as suggested by the severe sepsis study. The completion of the genomic sequences of many hosts and pathogens, as well as the recently published human haplotype map database, provide a tremendous opportunity to study whether some hemostatic factors are under positive selection. Studies on roles of variations of hemostatic factors in bacterial infections will not only identify novel therapeutic targets for treatment of infectious diseases but also provide individualized guidance for prevention and treatment of infectious diseases.

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