Review

Bench-to-bedside review: Functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis

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Abstract

The innate immune response system is designed to alert the host rapidly to the presence of an invasive microbial pathogen that has breached the integument of multicellular eukaryotic organisms. Microbial invasion poses an immediate threat to survival, and a vigorous defense response ensues in an effort to clear the pathogen from the internal milieu of the host. The innate immune system is able to eradicate many microbial pathogens directly, or innate immunity may indirectly facilitate the removal of pathogens by activation of specific elements of the adaptive immune response (cell-mediated and humoral immunity by T cells and B cells). The coagulation system has traditionally been viewed as an entirely separate system that has arisen to prevent or limit loss of blood volume and blood components following mechanical injury to the circulatory system. It is becoming increasingly clear that coagulation and innate immunity have coevolved from a common ancestral substrate early in eukaryotic development, and that these systems continue to function as a highly integrated unit for survival defense following tissue injury. The mechanisms by which these highly complex and coregulated defense strategies are linked together are the focus of the present review.

Keywords coagulation, disseminated intravascular coagulation, inflammation, sepsis, septic shock

Humoral and cellular elements of the innate immune system (complement components, mannose binding lectins, soluble CD14, defensins, antimicrobial peptides, neutrophils, monocyte/macrophage cell lines, natural killer cells) are recognized as the principal early responders following microbial infection. Perturbations of the clotting system frequently accompany systemic inflammatory states, and at least some of the elements of the coagulation system are almost invariably activated in patients with septic shock [1–3]. The simultaneous activation of the inflammatory response and the clotting cascade following tissue injury is a phylogenetically ancient survival strategy. The linkage between coagulation and inflammation can be traced back to the earliest events in eukaryotic evolution before the separation of plants and invertebrate animals from the evolutionary pathway that led toward vertebrate animal development.

Homologous structures of the Toll and IL-1 receptor domain are found in plants, where they function to activate antimicrobial peptides within plant cells in response to microbial invasion (for review [4,5]). The evolutionary linkage between coagulation and inflammation is perhaps best exemplified by the study of host defenses of the horseshoe crab (*Limulus polyphemus*). This ubiquitous crab commonly inhabits coastal marine waters in the temperate regions of the Northern Hemisphere. This animal has been invaluable in the study of ancestry of the coagulation cascades and antimicrobial defense mechanisms of the innate immune response.

APC = activated protein C; CRP = C-reactive protein; EPCR = endothelial protein C receptor; IL = interleukin; LBP = lipopolysaccharide-binding protein; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor-κB; PAI = plasminogen activator inhibitor; PAR = protease-activated receptor; TAFI = thrombin activatable fibrinolysis inhibitor; TF = tissue factor; TFPI = tissue factor pathway inhibitor; TLR = Toll-like receptor; TNF = tumor necrosis factor.

This invertebrate species possesses an open circulatory system (the hemolymph) and lacks differentiated, blood-forming elements such as neutrophils, erythrocytes and platelets. They have evolved a relatively simple but remarkably successful mechanism for defending the host after a breach of their integument (exoskeleton) by either trauma or infection [6,7]. The horseshoe crab has probably inhabited the earth largely unchanged from its current form for over 250 million years. Remarkably similar horseshoe crab ancestors can be found in the fossil record dating back to almost 1 billion years ago. There is suggestive biochemical evidence that endotoxin evolved and was expressed in cyanobacteria that existed on earth at least 2 billion years ago [8].

The innate immune system evolved to recognize highly conserved, simple but essential structures that are widely expressed within members of the Archea and Bacteria kingdoms, but are not found in multicellular, eukaryotic organisms [5]. Molecules such as lipopolysaccharide (LPS; also known as endotoxin), bacterial flagellin, peptidoglycan, and unmethylated CpG motifs of bacterial DNA are unique and essential structural elements of prokaryotic organisms [4]. The ability to discriminate rapidly between these non-self and self-molecules has an obvious survival advantage and forms the fundamental molecular basis for the innate immune defense strategy against microbial pathogens [7,9–12].

Any injury to the exoskeleton of the crab immediately jeopardizes the integrity of the internal milieu of the organism. Not only is there a real threat of loss of internal contents of the crab to the external environment, but there is also an omnipresent risk for entry of potentially pathogenic micro-organisms from the marine environment through the damaged protective crab shell. Both the loss of internal milieu through the crab's open circulatory system and contamination of its vital structures by microbial invaders threaten the survival of the entire arthropod organism.

In response to this threat, the horseshoe crab has evolved a rapid response system that begins with activation and degranulation of its sole circulating blood element, known as the hemocyte or amebocyte, at the site of local injury. The amebocyte simultaneously performs the dual functions of both platelets and phagocytic cells. The amebocyte will recognize the presence of bacterial LPS via its Toll receptors and engage micro-organisms by phagocytosis in an attempt to clear microbes from the site of injury. The primary molecular alarm signal that initiates this cellular host response is bacterial endotoxin [6]. Endotoxin induces degranulation and release of a complex series of soluble proteins from intracellular granules from amebocytes. These proteins work as a cascade system that terminates in the formation of an insoluble extracellular clot. This reaction occurs with such speed and reliability that it forms the basis for the widely used *Limulus* amebocyte lysate gelation reaction for endotoxin detection. The *Limulus* amebocyte lysate test remains the 'gold standard' for detection of endotoxin within biologic fluids [13].

This coagulation reaction serves two critical functions for the crab following tissue injury: it mechanically plugs up the physical damage to the integument of the animal, and it seals off the injured area from the remainder of the crab's open circulatory system. The animal will often clot off and sacrifice an entire extremity (they have seven more appendages to spare and they will grow back) and thereby avoid a potentially lethal systemic infection. The clot reaction walls off the injured site and contains the inevitable microbial contamination that occurs following a breach in the integument of this marine animal.

The clotting proteins of the crab provide an additional defense against microbial invasion. The *Limulus* coagulation cascade contains a regulatory protein known as *Limulus* anti-LPS factor, which recognizes and neutralizes bacterial LPS [14]. *Limulus* anti-LPS factor has antibacterial properties against Gram-negative bacteria, and forms the basic elements of a rudimentary innate immune defense within this phylogenetically preserved but remarkably successful invertebrate species [7,15].

The basic elements of clotting and inflammation have diverged in vertebrates into the platelets, neutrophils, macrophages, and other antigen-presenting cells, but the essential co-operation and interactions between clotting and inflammation are well preserved and readily demonstrable in human physiology today. Most of the inflammatory signals responsible for immune activation will also precipitate procoagulant signals to the coagulation system. As is discussed in considerable detail in the following sections, elements of the coagulation system feed back and rapidly upregulate innate immune responses. Many of the coagulation molecules and inflammatory molecules of the human innate immune system share structural homologies suggesting a common ancestral origin (e.g. CD40 ligand from platelets and the tumor necrosis factor [TNF] superfamily of proteins, and tissue factor [TF] homologies with cytokine receptors). Coagulation directly contributes to the systemic inflammation that characterizes severe sepsis [15–23].

It was recently shown that administration of a recombinant form of the endogenous anticoagulant activated protein C (APC) improves the outcome of patients with severe sepsis [24], confirming the therapeutic value of coagulation inhibitors in human sepsis. Importantly, this anticoagulant also significantly reduced circulating levels of IL-6 – a commonly measured inflammatory biomarker in septic patients. This verifies the intricate linkage between coagulation and inflammation in human sepsis, and indicates that the systemic inflammation of sepsis can be limited by the use of this natural anticoagulant.

Evolutionary biologists have observed that cascades of proteins that serve as precursor molecules with active and

inactive forms have evolutionary advantages in eukaryotic development [25]. These protein cascades provide sufficient flexibility and redundancy that mutations in these regulatory pathways may alter the expression of multiple enzyme systems. These mutations in cascades of regulatory proteins could be tolerated without the loss of the entire organism's viability. This permits phenotypic variation in enzyme systems within populations of metazoan life forms. These systems provide a substrate for what is termed 'evolvability' within complex multicellular organisms [25]. The more complex and the greater the need for longevity in vertebrate evolution, the more common and multifunctional these protein cascades become. Such protein signaling cascades are replete in the human coagulation system and in the innate immune response to invasive microbial pathogens. These evolutionary adaptations gave rise to the highly integrated clotting and inflammatory pathways in the human host response to tissue injury.

Functional interrelationships between clotting and the innate immune response

The close ancestral and functional linkage between clotting and inflammation is readily appreciated in study of human physiologic responses to a variety of potentially injurious stimuli. Many of the same proinflammatory stimuli that activate the contact system of the human clotting cascade also activate the phagocytic immune effector cells [2,3].

Pattern recognition molecules of the innate immune system function in a manner that is remarkably similar to that of contact factors of the intrinsic clotting system. The pattern recognition molecules of the innate immune defense system recognize surface features of microbial pathogens that differ from human cell membranes [4,5]. This results in the generation of a network of early host response signals that alerts the host to the presence of a potential microbial threat [12].

The contact factors of the intrinsic clotting system recognize damaged host cell membranes, foreign substances (particularly negatively charged molecules, including lipids such as bacterial LPS), and abnormalities along endothelial surfaces [1,2,26]. TF initiates the extrinsic pathway of blood coagulation, the primary pathway that is responsible for normal hemostasis. TF is expressed at high levels on vascular cells surrounding the endothelium [27]. Vascular damage leads to contact with these extravascular cells, with resultant rapid clot formation and cessation of blood loss. As is the case with the innate immune response, localized activation of the coagulation system and clot formation serves an important survival function to the host in the presence of a discrete traumatic injury.

De novo TF expression is responsible for triggering blood coagulation in response to systemic microbial invasion [1,28]. In this case, TF expression is induced on monocytes/macrophage systemically, but is rarely seen on endothelium [29]. Perhaps with localized infection the localized fibrin/platelet deposition might aid in walling off the infection, as seen with *Limulus* crabs. However, generalized intravascular coagulation (as is seen in the presence of invasive bloodstream infections) is clearly disadvantageous to the host, with consumption of clotting factors and widespread deposition of fibrin clots throughout the microcirculation [2,19–21].

The actions of the human coagulation system and the innate immune system are strikingly homologous in septic shock. Localized and controlled immune responses to discrete, focal infectious processes are clearly advantageous to the host. This contains the infection, eliminates the microbial pathogens, and initiates the tissue repair process. The survival advantage afforded by the immune response to localized infection becomes disadvantageous in the presence of systemic infection. The generalized systemic immune activation that follows bloodstream invasion participates in the genesis of widespread endothelial injury and diffuse tissue damage, culminating in lethal septic shock [30–32].

Humans are considered to be among the most sensitive of all mammalian species to the pathophysiologic effects to bacterial endotoxin [33], and the human clotting system is extremely efficient in responding to any form of vascular injury [19,20]. Selection pressures placed on the human genome over hundreds of thousands of years of early hominid evolution appear to have favored a vigorous early response to tissue injury and/or an infectious challenge. Our primate ancestors' relatively thin skin, in concert with the adoption of a predatory lifestyle only a few million years ago, would inevitably have led to an accumulation of frequent minor injuries and infections. This must have placed great demands on our innate immune defenses and clotting potential.

The recent acquisition of antimicrobial agents, immunizations, public sanitation, improved surgical techniques, and critical care units has changed the survival advantage that accrues from a potent immune defense system. We now find human populations in developed countries suffering from a dramatic increase in the incidence of chronic inflammatory (i.e. Crohn's disease, asthma) and immune-mediated disorders (i.e. type 1 diabetes mellitus, multiple sclerosis) as infectious diseases become less prevalent in modern societies [34,35].

It is now feasible to stabilize and successfully resuscitate patients with severe and very extensive injuries. Such patients would have had no chance for survival even a few generations ago. Systemic infections or major traumatic injuries that are routinely managed in modern critical care units would have represented a death sentence a century ago.

Currently, the same endogenous clotting and potent inflammatory processes that were so advantageous to our hominid ancestors often prove to be a liability in the intensive care unit patient with severe sepsis. Therapeutic approaches that limit the deleterious effects of excess clotting and inflammation

have become major research priorities in critical care medicine. The key element in this type of research is to limit the systemic inflammatory and coagulopathic damage, while retaining the benefits of controlled antimicrobial clearance capacity and localized clot formation [1,15,18].

Major elements of the human innate immune response

The innate immune system (monocyte/macrophage cell lines, neutrophils, natural killer cells, the alternative complement pathway, and other humoral elements of innate immunity) has evolved as an early, rapid response system to microbial invasion. Actions against the invading pathogens are either direct (e.g. phagocytosis and killing) or indirect, through release of cytokines or other stimulatory molecules that trigger the adaptive immune system by activating B and T cells.

The identification of infectious agents by means of conserved structural features through pattern recognition receptors is the central unifying concept of innate immunity [4,8]. The conserved components expressed by microbial pathogens that trigger the immune response are termed 'pathogen-associated molecular patterns'. The Toll-like receptors (TLRs), along with CD14 and its accessory molecules, are the major pattern recognition receptors that detect these pathogen-associated molecular patterns. The major microbial elements that are recognized by innate immune cells and their respective pattern recognition receptors are listed in Table 1.

Interleukin-1 receptor/Toll-like receptor superfamily and innate immunity

Several key molecules are involved in self/non-self recognition in the innate immune system. These include the serum complement [11], C-reactive protein (CRP) [10], mannose binding lectin [9], LPS-binding protein (LBP), and soluble CD14 [12]. The cell surface receptors include membrane CD14, the CD11–CD18 complex, and the TLRs on the surface of neutrophils and monocyte/macrophage cell types. According to our current understanding, the process of LPS recognition is initiated by binding of fragments of bacterial cell walls, and even whole bacteria, to LBP (a serum acute phase protein). Complexes of LBP and bacterial constituents may then easily bind to membrane CD14, a process that occurs much less effectively in the absence of LBP, although alternative mechanisms of LPS transfer to membrane CD14 may also exist [36].

Membrane-bound CD14, previously termed 'the endotoxin receptor', is a glycosyl phosphatidylinositol-anchored surface protein on myeloid cells. CD14 binds a wide array of microbial constituents in addition to bacterial endotoxin, such as peptidoglycan, lipoteichoic acid, and even fungal antigens [37,38]. CD14 is therefore a prototypical pattern recognition receptor [39]. Soon after the discovery of CD14 it became evident that the molecule is anchored to the cell membrane by a single covalent bound via its glycosyl phosphatidylinositol-linked tail, which lacks a membrane-spanning domain and is incapable of directly transmitting an intracellular signal. The molecular nature of the actual signal-transducing surface receptor was not discovered until 1998, with the identification of the TLRs [40].

The TLRs exist as a rather large family of type 1 transmembrane receptors. A total of 10 TLR open reading frames exist in the human genome. The gene products exhibit a number of structural and functional similarities. All TLRs express a series of leucine-rich repeats in their ectodomain, a transmembrane domain, and an intracellular domain that bears striking homology to the intracellular domain of the IL-1 type 1 receptor. This region of homology is known as the TIR (TLR IL-1 receptor) domain. When macrophages are activated by LPS, a complex of membrane CD14, an adapter protein known as MD2, and a TLR4 homodimer cluster on the cell surface in close proximity in 'raft'-like arrays [41].

In contrast to CD14, TLRs have greater ligand specificity for the microbial structures and they can detect and discriminate between types of bacteria and other microbial components. Gram-positive bacterial components such as peptidoglycan and lipopeptides are recognized by heterodimers consisting of TLR2 in combination with TLR6 (for peptidoglycan) or TLR1 (for bacterial lipopeptides). Most forms of Gram-negative bacterial LPS are specifically recognized by TLR4. TLRs may detect additional microbial structures in a CD14-independent manner, including the following: flagellin (TLR5); prokaryotic unmethylated CpG motifs in bacterial DNA (TLR9); mycobacterial lipoarabinomannan (TLR2); fungal constituents (TLR6 and TLR2 heterodimers); and even double-stranded viral RNA (TLR3). These ligand–receptor interactions are summarized in Table 1. The intracellular events that follow engagement of each TLR by its cognate natural ligand are increasingly being recognized and consist of release of a specific series of tyrosine kinases and mitogen-activated protein kinases (MAPKs) that result in activation of transcriptional activators such as nuclear factor-κB (NF-κB) and activator protein-1 (for detailed review [5,42]). The principal elements of the signaling events that follow engagement of the human TLRs are shown in Fig. 1.

Major elements of the human coagulation system

This subject was reviewed in considerable detail recently [1–3,43,44], and the major clotting parameters that interact in sepsis are shown in Fig. 2. The extrinsic pathway (TF pathway) is the primary mechanism by which thrombin is generated in sepsis, hemostasis and thrombosis. The intrinsic cascade (contact factor pathway) primarily serves an accessory role in amplifying the prothrombotic events that are initiated in sepsis. Thrombin, factor Xa, and the TF–factor VIIa complex directly activate endothelial cells, platelets and white blood cells, and induce a proinflammatory response. The inflammatory reaction to tissue injury activates the clotting

Table 1

Pattern recognition receptors of the innate immune response and their major known natural ligands

Receptor or related structure	Cell type or soluble factor	Examples of known natural microbial ligands
CD14	Myeloid cells and soluble forms	PAMPs from bacterial, fungal, and mycobacterial antigens
Mannose binding lectin	Soluble factor	Binds to mannosides found on bacteria and fungi; activates complement and opsonin for neutrophils
C-reactive protein	Soluble protein	Opsonin for Gram-positive bacteria
LPS-binding protein	Soluble protein	Binds to LPS in Gram-negative bacteria and lipoteichoic acid Gram-positive bacteria
C′3, alternative complement	Soluble proteins	Polysaccharide capsules of bacteria, fungi
MD1	B cells	Coreceptor for LPS on cell surface of B cells
MD2	Myeloid cells	Coreceptor for LPS on macrophages, neutrophils
TLR1	Myeloid cells	Lipopeptide, lipoteichoic acid, LPS of leptospirosis
TLR2	Myeloid cells	Peptidoglycan, lipopeptide, lipoarabinomannan, fungal cell wall components, LPS of leptospirosis
TLR3	Myeloid cells	Double-stranded viral RNA
TLR4	Myeloid cells	LPS, respiratory syncytial virus proteins
TLR5	Myeloid cells	Flagellin from Gram-positive or Gram-negative bacteria
TLR6	Myeloid cells	Zymosan (fungal constituents) along with TLR2
TLR9	Dendritric cells, B cells, epithelial cells	Unmethylated CpG motifs in prokaryotic DNA

LPS, lipopolysaccharide; PAMP, pathogen associated molecular pattern; TLR, Toll-like receptor.

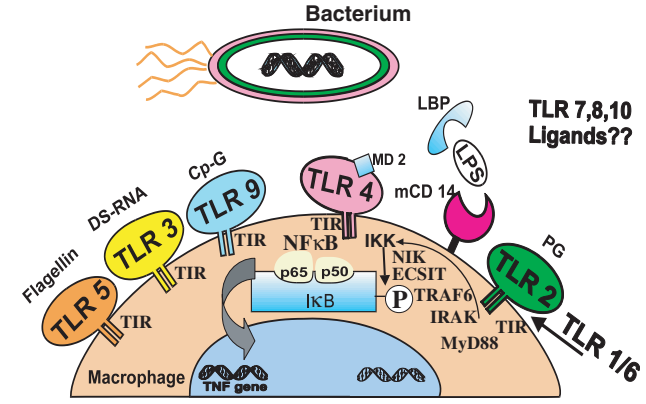
system, inhibits the endogenous anticoagulants, and attenuates the fibrinolytic response. The net effect within the microcirculation is a procoagulant state, which has major therapeutic implications [16,17,24,45].

It was traditionally thought that the contact factors, factor XII, factor XI, prekallikrein, and high-molecular-weight kininogen were the primary activators of the clotting cascade in sepsis. It is clear that contact factors can be activated by cell wall components found on both Gram-positive and Gram-negative bacteria. The negatively charged bacterial molecule LPS is the prototypical microbial inducer of the coagulation cascade. Activation of these coagulation factors generates a factor X converting complex consisting of factor IXa and the acceleration factor VIIIa, resulting in activation of the common clotting pathway at the level of factor X activation. The cascade then follows via conversion of prothrombin to thrombin by activated factor X in the presence of the accelerating cofactors, namely factor Va, negatively charged phospholipid, and calcium. Thrombin generation is immediately followed by fibrin monomer production through degradation of fibrinogen with subsequent polymerization to fibrin clots and stabilization of fibrin by the action of factor XIIIa, an enzyme that is generated by thrombin activation of factor XIII.

It is now recognized that this classical view of the coagulation activation via the contact factor system is not the primary pathway of thrombin generation and fibrin generation in most patients with sepsis. The extrinsic pathway of coagulation is the essential pathway of clot formation in sepsis (see below). Studies in which sublethal doses of endotoxin were administered to human volunteers [1,11,46] revealed no evidence of contact factor activation, despite thrombin generation as measured by thrombin–antithrombin complexes and prothrombin fragment 1.2 generation. Prothrombin fragment 1.2 is a reliable measure of ongoing thrombin generation because this peptide is released from prothrombin during active thrombin generation. Moreover, the antibodies that specifically block the intrinsic clotting system do not diminish the frequency of thrombin generation in experimental animal studies of sepsis [46]. These antibodies did, however, prevent the contact pathway generation of bradykinin. Thus, although the contact pathway is activated in experimental sepsis and contributes to vasodilatation, it does not contribute significantly to thrombin generation [2,26].

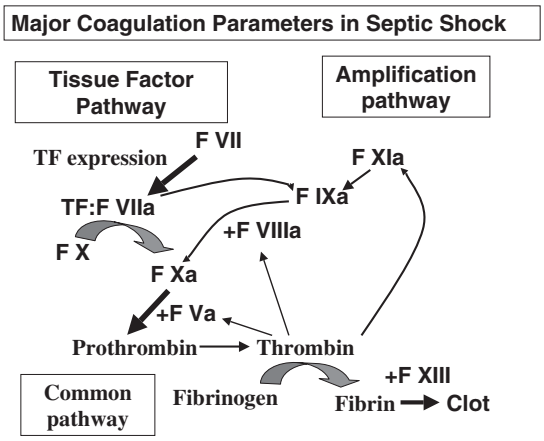
It should be noted that, during actual human septic shock, contact factor activation might in fact occur, as indicated by systemic release of bradykinin from high-molecular-weight kininogen. Bradykinin is a potent vasoactive substance that may contribute to the hypotension and diffuse capillary leak that typifies septic shock [26]. The intrinsic pathway can also be activated by thrombin itself, and this system may function as an amplification pathway in sepsis-induced disseminated

Figure 1



The human Toll-like receptors (TLRs) and their known ligands. CpG, cytosine-phosphoryl-quanine; ECSIT, evolutionarily conserved signaling intermediate of Toll; IκB, inhibitory kappaB; IKK, IκB inducing kinase; IRAK, IL-1 receptor associated kinase; LBP-lipopolysaccharide-binding protein; LPS, lipopolysaccharide; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κ for B cells; NIK, NF-κB inducing kinase; PG, peptidoglycan; TIR, Toll IL-1 receptor domain; TRAF6, tumor necrosis factor receptor associated factor-6.

Figure 2



The major coagulation factors and the pathways of coagulation activation in sepsis. TF, tissue factor; t-PA, tissue-type plasminogen activator.

intravascular coagulation [47]. Recent evidence indicates that the intrinsic clotting system is frequently activated in experimental and perhaps clinical streptococcal toxic shock [48].

Current evidence indicates a dominant role of the TF pathway (extrinsic pathway) for coagulation activation in sepsis (Fig. 2). TF is not normally expressed within the endovascular system, and resides on vascular smooth muscle cells and fibroblasts within the adventitia around blood vessels. This arrangement is ideal under physiologic conditions because TF only becomes exposed to other clotting components after injury to the vessel wall when blood is extravasated into the interstitium [47]. TF expression is upregulated on monocytes/macrophages and to a limited extent, if at all, on endothelial cells [27] following exposure to proinflammatory mediators such as endotoxin, CRP, IL-1, and IL-6 [1,2,49,50]. Soluble TF, defined as TF activity resident in plasma, may also be found in the circulation in patients with sepsis. Much of the soluble TF may be resident in microparticles released from activated or damaged mononuclear cells [51]. TF expressed within the intravascular space will bind to circulating factor VII, resulting in a TF–factor VIIa complex [52]. TF–factor VIIa complexes can directly activate the common pathway of coagulation by converting factor X to factor Xa. Factor Xa in the presence of factor Va forms a prothrombin-converting complex, resulting in thrombin formation and subsequent generation of a fibrin clot [2,53]. TF–factor VIIa complexes may also activate the intrinsic clotting system by converting factor IX to IXa, which, in the presence of factor VIIIa, can also activate factor X and result in the generation of a fibrin clot. This latter pathway appears to be important because the TF–factor VIIa complex is rapidly inactivated by tissue factor pathway inhibitor (TFPI) once traces of factor Xa are formed (See the section Tissue factor pathway inhibitor, below).

The contact factors of the intrinsic pathway play an important accessory role as an amplification loop in sepsis once the TF pathway activates coagulation. Thrombin generation feeds back at the level of factor XI and, to a lesser degree, factor VIII and factor V, to promote factor X conversion and thrombin generation via the contact factor system. The contact factor pathway also functions to activate the fibrinolytic system, along with the proinflammatory cytokine TNF [46,54].

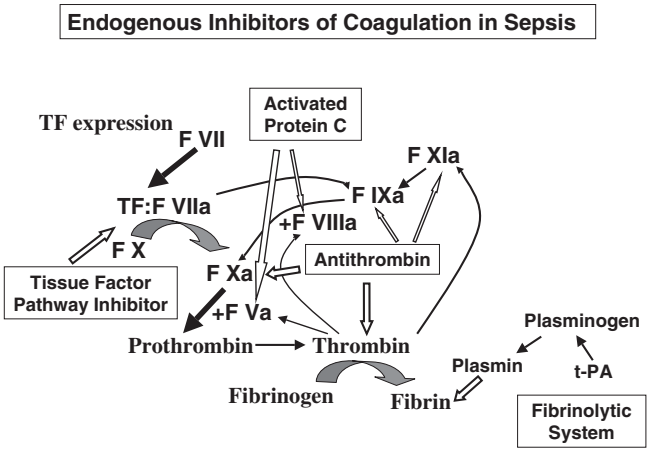
Endogenous mechanisms to prevent thrombus formation

There are four major systems that minimize thrombus formation in humans (Fig. 3). These include the fibrinolytic system, antithrombin, TFPI, and the protein C–protein S–thrombomodulin pathway. Depletion of these systems contributes to the consumptive coagulopathy and/or microvascular thrombosis of sepsis [1,2].

Fibrinolytic system

The fibrinolytic system is rapidly activated by proinflammatory cytokines, particularly TNF, in the early phases of sepsis [16,18]. This essential activity is initiated when plasminogen is converted into the potent, broad-spectrum protease plasmin. Plasmin degrades fibrin, fibrinogen, the acceleration factors V and VIII, and probably other substrates as well [55]. In most septic patients generation of plasmin is abruptly downregulated by simultaneous increase in the levels of

Figure 3



The principal coagulation regulatory pathways and their sites of action. TF, tissue factor; t-PA, tissue-type plasminogen activator.

inhibitors of fibrinolysis [54], including plasminogen activator inhibitor (PAI)-1 and PAI-2 [16,54,56–58]. Intravascular fibrinolysis is principally mediated by the actions of tissue-type plasminogen activator on plasminogen, and its primary inhibitor is PAI-1. Extravascular clots (e.g. fibrin deposition within the alveoli in acute respiratory distress syndrome, or inflammatory foci in tissues) are primarily degraded when urokinase-type plasminogen activator induces plasmin formation. PAI-2 inhibits the activity of urokinase-type plasminogen activator in the extravascular space [55].

Thrombin activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase B that is rapidly activated by the thrombin–thrombomodulin complex. The TAFIa that is generated removes basic lysine and arginine residues from the carboxyl terminus of peptides and proteins. In the case of fibrin, removal of carboxyl-terminal lysine residues renders the fibrin less sensitive to lysis by decreasing the ability of plasminogen and tissue-type plasminogen activator to bind to the fibrin, a step that facilitates clot lysis. Inhibition of thrombin generation by anticoagulants such as the APC–protein S complex prevents TAFI generation from its inactive precursor [56–58]. Genetic polymorphisms that lead to excess expression of PAI-1 [59] or TAFI [60] may place certain septic patients at greater risk for diffuse thrombosis and mortality because excess levels of these fibrinolysis inhibitors attenuate the fibrinolytic system. Although TAFI was named for its unquestionable ability to inhibit fibrinolysis, recent studies have suggested that a comparable if not more important function of TAFIa may be in the control of vasoactive substances (see the section on Activated protein C, below).

Tissue factor pathway inhibitor

TFPI is a 42-kDa protein that consists of three closely linked Kunitz domains [61,62]. These domains allow TFPI to function by a unique mechanism. Factor Xa generated by the TF–factor VIIa complex binds very tightly to and inactivates factor Xa. By virtue of the ability of factor Xa to bind to negatively charged phospholipids, the resultant complex interacts with damaged cells, raising the local concentration of TFPI. The TFPI–factor Xa complex then binds to the TF–factor VIIa complex. The latter interaction is of lower affinity and occurs poorly in the absence of the concentrating effects that result from the formation of the TFPI complex with factor Xa. Because this inhibitor rapidly inhibits factor VIIa bound to TF once the first factor Xa molecules are formed, the alternative activation of factor IX by the TF–factor VIIa complex becomes critical to thrombin generation and hemostasis. At therapeutic levels, TFPI anticoagulates blood both by direct inhibition of factor Xa and by the factor Xa dependent inhibition of the TF–factor VIIa complex.

The dynamics of TFPI activity in the microcirculation are rather complex and vary according to the amount of TFPI bound to endothelium or stored in endothelial vacuoles [63]. TFPI levels bound to lipoprotein and in platelets, and circulating TFPI that may be present in active form or less active cleaved TFPI [61,64]. The less active cleaved form of TFPI results from cleavage by neutrophil elastase or other serum proteases that are generated in severe sepsis. Most clinical assay systems are not able to discriminate between inactive cleaved TFPI and fully active TFPI [61]. These technical problems, along with the very low (nanogram range) quantities that are measurable in the circulation, have rendered TFPI levels in human sepsis difficult to measure and interpret [61,65]. Perhaps more important is that only a small amount of the total TFPI circulates in the blood. Heparin administration can elevate plasma levels of TFPI by about 10-fold, presumably reflecting release of bound or stored endothelial cell TFPI [62]. Because the vast majority of the endothelium is in the microcirculation, these findings indicate that the highest levels of TFPI are also found in the microvasculature and suggest that this inhibitor plays a key role in the regulation of microvascular thrombosis.

Consistent with a critical role played by TFPI in regulating microvascular thrombosis, genetic deletion of the TFPI gene in mice results in early embryonic lethality and microvascular thrombosis [66]. Furthermore, inhibiting TFPI with antibodies exacerbates the response to endotoxin infusion in experimental rabbit models of sepsis [67].

Certainly, however, both experimental and clinical evidence indicates that functionally active TFPI levels are inadequate within the microcirculation to prevent ongoing coagulation and organ dysfunction in sepsis. Exogenously added TFPI has been shown to reduce inflammatory [65,68] and coagulation activities [69,70] in experimental models of sepsis, and to improve outcomes in septic animals. These experimental findings form the therapeutic rationale for recombinant TFPI therapy, which is a logical strategy in clinical sepsis. Regret-

tably, a recently completed, large, phase 3 international sepsis trial with TFPI treatment was apparently unable to demonstrate a benefit from treatment. The details of that study are not available at present, pending the publication of the final study findings in the near future.

Antithrombin

The anticoagulant actions of antithrombin (formerly referred to as antithrombin III) are well known and relate to its ability to function as a potent endogenous serine protease inhibitor. Antithrombin is a hepatically synthesized plasma protein that is activated by the process of allosteric activation by heparin and related heparans. Specific polysulfated pentasaccharides, which are found in repeating units in glycosaminoglycans and mucopolysaccharides, are necessary to bind to a highly basic, central domain in antithrombin. A conformational change takes place in antithrombin following interactions with these acidic pentasaccharide moieties, bringing a critical arginine residue at position 393 to link covalently within the active site of serine proteases, thereby accelerating the inactivation of these proteases [71,72]. The conformational change in antithrombin induced by heparin is only part of the heparin mechanism, however. For heparin to function as an efficient stimulator of thrombin inhibition, higher molecular weight forms of heparin are needed. A higher molecular weight would allow heparin to form a bridge between antithrombin and thrombin. Heparins that are too small to form this bridge have almost no effect on thrombin inhibition by antithrombin but retain the ability to inactivate factor Xa [73].

Many of the clotting factors and regulators of the coagulation system are serine proteases, including thrombin, factor X, components of the contact system, and TF–factor VIIa–heparin complexes [74,75]. The broad substrate enzymatic activity of this plasma protease inhibitor allows antithrombin to play a central role in the regulation of coagulation. Antithrombin is rapidly consumed in sepsis by covalent linkage and clearance, along with the activated clotting factors [72]. Antithrombin levels are further diminished by enzymatic cleavage by neutrophil elastase production [76] and by diminished hepatic synthesis during sepsis [77]. Loss of anticoagulant activity as a result of reduced antithrombin levels participates in the generation of the prothrombotic state that characterizes septic shock [72,78,79].

In the absence of heparin, antithrombin binds to specific pentaccharide-bearing glycosaminoglycans on the cell surface of endothelial cells, such as heparan sulfate. When in contact with endothelial cells, antithrombin exerts both local anticoagulant and anti-inflammatory activities [80–82]. This is mediated in part by antithrombin-mediated induction of prostacyclin synthesis by endothelial cells. Prostacyclin is a potent antiplatelet agent that inhibits platelet aggregation and attachment. Prostacyclin also inhibits neutrophil–endothelial cell attachment and attenuates IL-6, IL-8, and TNF release by endothelial cells [82–84].

It has recently been demonstrated that antithrombin has additional anti-inflammatory effects via direct binding to neutrophil, lymphocyte, and monocyte cell surface receptors such as syndecan-4 [85–88]. Antithrombin reduces expression of IL-6 and TF, and inhibits of activation of the transcription factor NF-κB in LPS-stimulated monocytes and endothelial cells [89,90].

Antithrombin reduces chemokine (IL-8)-induced chemotaxis of neutrophils and monocytes in experimental systems. This may be mediated by a reduction in chemokine receptor density on leukocyte cell surfaces after binging to antithrombin. This direct inhibitory effect is blocked by heparin and synthetic pentasaccharides via competitive inhibition against antithrombin binding to sydecan-4 [85,86].

These anti-inflammatory activities are observed *in vivo* in a number of animal systems in which attenuation of white cell–endothelial cell interactions have been demonstrated by intravital microscopy [71,91,92]. The administration of antithrombin to LPS-challenged animals significantly reduced the interaction of inflammatory cells with the vessel wall (characterized by rolling, sticking, and transmigration events), thereby limiting capillary leakage and subsequent organ damage. Recently, Hoffman and coworkers [92] have confirmed these anti-inflammatory activities in a hamster model that quantifies functional capillary density *in vivo*. White cell adherence and loss of functional capillary density was rapidly induced by LPS, and this loss of microcirculatory surface was inhibited by therapeutic doses of antithrombin. Antithrombin-mediated preservation of functional capillary density is completely prevented by unfractionated or low-molecular-weight heparin. These anti-inflammatory actions have been demonstrated in a number of experimental systems [80,83,84, 92–97] and are presumably physiologically important within the microcirculation in human sepsis as well [72].

This may provide a partial explanation for the results of a recent phase 3 clinical trial with high-dose antithrombin in severe sepsis [98]. No overall benefit was found by administration of 30 000 IU of plasma-derived antithrombin over 4 days in that large international trial conducted in 2314 patients (38.9% antithrombin versus 38.7% placebo; not significant). It was observed that a prespecified subgroup of patients who received no heparin (30% of the overall study population) appeared to derive some modest benefit from antithrombin (15% relative risk reduction in mortality after 90 days; $P < 0.05$). These patients might have derived long-term benefits with respect to morbidity and quality of life indices as well [99].

The subgroup of patients who received heparin (up to 10 000 units/day, as allowed by the study protocol) experienced no improvement in outcome with antithrombin therapy but exhibited a significantly greater risk for hemorrhage than did placebo-treated patients (10.9% with antithrombin versus

6.2% in the control group; $P<0.01$). The use of concomitant heparin with antithrombin in that study might have blocked any potential, salutary, anti-inflammatory effects of antithrombin within the microcirculation, and this combination clearly exacerbated the risk for bleeding in severely septic patients [98].

Activated protein C

The APC pathway of anticoagulation is a classic negative feedback loop initiated by thrombin-dependent generation of the anticoagulant APC. The vitamin K-dependent protein C zymogen is transformed into APC by the proteolytic cleavage of 12 amino acids from the amino terminus of the heavy chain of protein C. This activation step is catalyzed very slowly by thrombin itself. Rapid activation of protein C occurs along the luminal surface of capillary endothelial cells. Thrombin is first complexed with its specific, membrane-bound, protein receptor, thrombomodulin. Once bound to thrombomodulin, thrombin is incapable of binding to fibrinogen for conversion to fibrin, can no longer activate platelets, and loses its pro-coagulant activity [21,22]. The thrombin–thrombomodulin complex retains a capacity to bind to its other substrate, protein C, and the rate of protein C activation relative to thrombin alone is increased about 1000-fold. Thrombin now becomes an anticoagulant enzyme converting the inactive precursor protein C to APC.

APC is a potent serine protease that, in comparison to other serine proteases (which usually have a half-life of seconds), has a relatively long elimination half-life from the plasma of approximately 15–20 min [18,22]. Feedback inhibition of new thrombin generation by APC is mediated by proteolytic degradation of the acceleration coagulation factors Va and VIIIa. APC activity is facilitated several fold by reversible binding to another hepatically synthesized, vitamin K-dependent protein known as protein S. This 'accessory' protein associates with APC only in its free circulating form; protein S bound to C4b-binding protein from the complement system cannot bind to APC [2,22,23].

In addition to inhibition of fibrin formation, APC also promotes fibrinolysis *in vitro* by inhibiting two important inhibitors of plasmin generation, namely PAI-1 [18,54] and TAFI [56–58]. This profibrinolytic activity of APC is not shared by antithrombin [1,2]. APC actually binds to the active site of PAI-1 and as such blocks the serine protease inhibitor actions of PAI-1 [2,100,101]. The reaction of APC with PAI-1 is relatively slow, but the rate is enhanced dramatically by vitronectin [102], raising the possibility that the profibrinolytic effects of APC might center around cells such as platelets that can release vitronectin. It has been speculated that these profibrinolytic activities of APC might have significantly contributed to the therapeutic efficacy observed in the recent phase 3 trial with recombinant human APC (drotrecogin alfa [activated]) in human sepsis [24]. The clinical relevance of this activity of APC remains to be convincingly demonstrated.

As discussed previously, TAFI is activated by the thrombin–thrombomodulin complex and this activation probably occurs in the microcirculation. TAFIa has been shown to inhibit fibrinolysis [57]. Inhibitors of thrombin formation would therefore inhibit TAFI activation and presumably facilitate clot lysis. TAFIa, however, is a carboxypeptidase with broad substrate specificity and a preference for removal of carboxyl-terminal arginine residues [103]. Removal of carboxyl-terminal arginine residues is a major mechanism for inactivation of vasoactive peptides. It was recently proposed that TAFIa is the major inhibitor of complement anaphylatoxin C5a. Because both prothrombin activation and complement activation occur in severe sepsis, it is not surprising that key regulatory mechanisms that are involved in controlling coagulation might also control complement. Inactivation of C5a would be expected to decrease neutrophil chemotaxis and systemic vasodilatation [103]. This is another example of the close interrelationship between clotting regulators and innate immune reactions. A summary of inflammatory reactions to the procoagulant and loss of anticoagulant activity found in sepsis is provided in Table 2.

APC has direct anti-inflammatory effects in experimental studies that are independent of the antithrombotic actions of this endogenous anticoagulant (for review [104]). APC binds to specific receptors on endothelial cells and white cells. The only receptor isolated and characterized to date is known as endothelial protein C receptor (EPCR) [18,105]. This APC–EPCR complex can translocate from the plasma membrane to the nucleus, where it presumably alters gene expression profiles. Other evidence suggests that APC cleaves a receptor on the cell surface [106,107], and in some cases this appears to be EPCR dependent [108]. APC bound to EPCR has also been shown to cleave protease-activated receptor (PAR)-1 and PAR-2 [109], but how this facilitates the *in vivo* anti-inflammatory effects observed with APC [104] remains to be determined. *In vivo*, the anti-inflammatory effects of APC that are independent of its anticoagulant effects include inhibition of neutrophil adhesion, decreased TNF elaboration, and decreased drops in blood pressure (for review [104]). APC has multiple effects in tissue culture systems, including limitation in NF-κB-mediated proinflammatory activity [110], attenuation of inflammatory cytokine and chemokine generation [23], and upregulation of antiapoptotic genes of the Bcl-2 family of homologs [111].

Endothelial cells are relatively resistant to apoptosis as a result of the constitutive synthesis of a number of antiapoptotic proteins [112]. Microbial mediators, such as bacterial LPS, can overcome the inhibition of apoptosis within endothelial cells. APC protects endothelial cells from apoptosis in experimental systems. It remains to be demonstrated whether this activity is relevant to the protective effects of APC in human sepsis.

In experimental studies and in human sepsis circulating blood levels of protein C rapidly decline, with loss of this important

Table 2

The inflammatory effects of coagulation and loss of anticoagulants

Coagulation parameter	Proinflammatory effects
Thrombin generation	Promotes cytokine and chemokine synthesis (IL-6, IL-8) via PARs, P-selectin, E-selectin and PAF expression, which facilitates neutrophil–endothelial cell interactions, bradykinin and histamine release
Factor Xa and TF–factor VIIa complex generation	Promotes cytokine and chemokine synthesis (IL-6, IL-8) via PAR-1 and PAR-2
Reduced antithrombin	Results in the loss of prostacyclin synthesis by endothelial cells, increased cytokine synthesis, increased leukocyte adherence and chemotaxis
Reduced protein C/protein S activity	Results in increased E-selectin expression, increased cytokine generation and neutrophil adherence; promotes apoptosis of endothelial cells
Reduced TFPI activity	Results in loss of regulation of cytokine synthesis within microcirculation
Platelet activation	Platelet derived P-selectin promotes neutrophil adherence, neutrophil–endothelial cell interactions; platelet CD40 ligand promotes endothelial cell chemokine and adhesion molecule expression; activated platelets secrete chemokines and IL-1β
Intravascular fibrin deposition	Neutrophil and monocyte adherence
Reduced TM expression on endothelial cells	Loss of TM lectin domain activity that inhibits neutrophil–endothelial cell adherence may promote neutrophil binding

IL, interleukin; PAF, platelet-activating factor; PAR, protease activated receptor; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin.

coagulation inhibitor function [113,114]. Protein S functional levels also decrease. There is evidence that peripheral conversion of protein C to APC is impaired as a result of diminished expression or cleavage of EPCR [115–117] and thrombomodulin [118] in the microcirculation. Soluble thrombomodulin is readily measurable in the circulation of septic patients [119,120], and biopsies of blood vessels in patients with meningococcal disease confirm the loss of thrombomodulin and EPCR expression along endothelial surfaces during severe sepsis [121]. The extent to which these protein C activators are downregulated in severe sepsis appears to vary widely [122]. These findings provide the therapeutic rationale for the administration of APC in severely septic patients [24].

In the phase 3 clinical trial [24], recombinant human APC (drotrecogin alfa [activated]), administered by continuous infusion at a dose of 24 μg/kg per hour for 4 days, reduced the mortality rate from 30.8% in the placebo group ($n=840$) to 24.7% in the recombinant human APC group ($n=850$; $P=0.005$). This indicates an absolute reduction in mortality rate of 6.1% and a relative risk reduction of 19.4% associated with treatment with drotrecogin alfa (activated).

In experimental models of sepsis, soluble thrombomodulin has been shown to have both anticoagulant and anti-inflammatory activity. Much of the anti-inflammatory activity was believed to be mediated by protein C activation. Recently, the lectin domain of thrombomodulin was shown to have direct anti-inflammatory activity by reducing adhesion molecule expression and inhibiting MAPK and NF-κB pathways, thereby inhibiting the ability of leukocytes to bind to activated endothelium *in vivo* [123]. As mentioned above, infusion of thrombomodulin would be expected to inhibit the activities of vasoactive substances and to inhibit thrombin clotting activity directly. These newly identified functions of thrombomodulin suggest that it might be a good therapeutic target in severe sepsis, but one that might require protein C supplementation to be effective.

EPCR, the other receptor that is involved in protein C activation, appears to have direct anti-inflammatory activity also. Soluble EPCR, which is released in response to thrombin activation of the endothelium [124], binds to proteinase-3, a serine protease released from activated neutrophils. This complex in turn binds to Mac-1 [125], which is an important integrin involved in tight neutrophil adhesion. Of interest, proteinase-3 is the autoantigen in Wegener's granulomatosis. It appears that soluble EPCR binding to this complex results in inhibition of tight neutrophil adhesion.

In considering therapy with protein C pathway components, protein C supplementation is an obvious possibility, especially because protein C levels are decreased, sometimes severely, in severe sepsis. There are several anecdotal reports of success in treating patients with severe sepsis with protein C [126–128]. The disadvantage of this approach is that the protein C activation complex may be downregulated severely in some patients with severe sepsis. The advantage, however, is that protein C activation is tightly regulated and ceases locally as soon as thrombin formation is controlled.

Table 3

Procoagulant effects of inflammatory mediators

Inflammatory mediator	Procoagulant effects
Proinflammatory cytokines	Increased TF expression on endothelium, monocytes; decreased TM and endothelial protein C receptor; increased PAI-1; release of TFPI from endothelium with loss of activity
Complement components	Decreased C1-esterase inhibitor leads to loss of contact factor regulation; damaged cell membranes promote procoagulant activity on cell surfaces of endothelial cells
Acute phase proteins	Increase in clotting factor synthesis; decrease in synthesis of antithrombin; α_1-antitrypsin decreases APC and cleaves TFPI; CRP promotes TF expression; C4b-binding protein binds to protein S and limits protein C activity
Neutrophils	Elastase destroys antithrombin, C1-inhibitor, thrombomodulin, and cleaves TFPI; intravascular neutrophil–platelet aggregates occlude capillary beds
Activated monocytes	Upregulation of TF expression; IL-6 and TNF synthesis promote acute phase proteins with procoagulant activities; release of microvesicles with TF in circulation
Activated endothelium	P-selectin promotes platelet aggregation, procoagulant surface upregulation of TF; PAF expression stimulates platelets; shedding of glycosaminoglycans limits antithrombin binding; loss of TM and EPCR expression limits APC synthesis

APC, activated protein C; CRP, C-reactive protein; EPCR, endothelial protein C receptor; PAF, platelet activating factor; PAI, plasminogen activator inhibitor; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TNF, tumor necrosis factor.

Thus, protein C supplementation has the advantage of generating high levels of APC locally. In addition, because both protein C and APC bind to EPCR, activation of endogenous protein C allows selective loading of EPCR with APC, which should serve to amplify APC-dependent, receptor-mediated anti-inflammatory activities of APC.

The mechanisms by which inflammatory responses promote coagulation

Inflammation promotes coagulation via a large number of molecular and cellular mechanisms (Table 3). Perhaps the most direct mechanism responsible for the procoagulant activity of the inflammatory response is through the generation of proinflammatory cytokines [1,2]. A number of cytokines, particularly IL-6, increase the expression of TF on endothelial surfaces and monocytes [1,129,130]. The TF pathway, also referred to as the extrinsic clotting cascade, is the principal activator of clotting in the presence of systemic inflammation and generalized infection.

TNF and IL-1β, which are major cytokines in the pathogenesis of septic shock, also inhibit the expression of EPCR [117] and thrombomodulin on endothelial cells [118]. Thrombomodulin is primarily located on the endothelial surfaces of capillaries within the microcirculation, whereas EPCR is principally located on endothelial surfaces of larger vessels on small arteries and arterioles [119]. Together, these molecules facilitate the generation of APC by bringing thrombin into direct contact with protein C. Loss of thrombomodulin and EPCR via proinflammatory cytokine production impairs conversion of protein C to APC [121]. The reduced levels of protein C and APC contribute toward the procoagulant state that typifies severe sepsis [1,2,24,131,132]. TNF is a potent inducer of fibrinolysis in sepsis through the synthesis of tissue-type plasminogen activator; however, fibrinolytic activity is rapidly inhibited by the almost simultaneous production of increased levels of PAI-1 [54]. This culminates in a pathophysiologic state of cytokine-induced activation of coagulation, diminished anticoagulant activity, and suppressed fibrinolysis, with widespread thrombin generation and intravascular fibrin deposition.

Activated neutrophils along endothelial surfaces release the broadly reactive proteolytic enzyme elastase that destroys antithrombin and C1-esterase inhibitor and releases thrombomodulin in a less active form [94,128]. Both of these regulatory proteins are important endogenous inhibitors of the coagulation system. C1-esterase inhibitor is the major regulator of the intrinsic or contact factor pathway of coagulation [1,2,131]. This pathway remains of significance in sepsis despite the fact that the TF pathway is the initiator of clotting in systemic inflammatory states. Factor IX of the intrinsic system can be activated by the TF–factor VII complex, and thrombin can activate factor XI and to some degree factors VIII and V. This accessory system serves to amplify and maintain coagulation in sepsis [132].

The acute phase protein CRP upregulates TF, whereas another acute phase protein, namely α_1-antitrypsin, inhibits APC. Both of these actions result in a procoagulant state [43,50,53,120]. CRP synthesis has activities other than the promotion of TF expression and coagulation activation. It also promotes complement activation via the classical complement pathway [10]. Complement activation in response to inflammatory stimuli depletes several control elements of the coagulation system. Complement components activate neutrophils, promote neutrophil chemotaxis, stimulate cytokine synthesis, and contribute to increased capillary permeability

and systemic hypotension [2,11,43]. Contact factor activation generates systemic synthesis of bradykinin, resulting in systemic hypotension and tissue hypoperfusion. Moreover, focal regions of tissue ischemia as a result of intravascular clot formation stimulate an intense inflammatory response [1,19,20].

The acute phase complement component C4b-binding protein is upregulated in inflammation, and binds and inactivates protein S. Protein S is an endogenous coagulation inhibitor that enhances the activity of APC as an inhibitor of factors Va and VIIIa [2,100]. A summary of some of the procoagulant effects that accompany the acute inflammatory response is provided in Table 3.

Role of the coagulation pathways in systemic inflammation

Intravascular thrombin generation is highly inflammatory within the microcirculation via interaction with specific receptors on platelets, endothelial cells and white blood cells known as PARs [133,134]. The PARs are typical seven-transmembrane, G-protein-linked receptors that differ from other receptors in that their ectodomain possesses a sequestered internal ligand that is tethered to the amino-terminus of its extracellular domain. Thrombin cleaves the amino-terminus of the PAR, allowing the internal ligand to autoactivate the receptor.

There are four known PARs in human biology named PAR-1–4. PAR-2 is responsive to trypsin. The TF–factor VIIa complex may also activate PAR receptors [135], which may be mediated by PAR-2. Factor Xa can also directly activate cells via PAR-1-mediated cell activation [135]. Activation of cells mediated by thrombin and other coagulation factors increases proinflammatory cytokine synthesis and calcium flux, alters intracellular signaling cascades such as the MAPK pathway, and induces nitric oxide synthesis [17,18]. Thrombin also stimulates production of platelet-activating factor [136]. Platelet-activating factor is a potent neutrophil-activating substance, especially when the neutrophils are tethered to P-selectin, a molecule that is expressed on endothelium and platelets in response to thrombin [137,138].

Activated platelets contribute to local inflammatory processes at the site of clot formation by a number of mechanisms. Platelets can secrete chemokines and IL-1, which activate white cells and promote neutrophil and monocyte adherence [139]. Platelets express P-selectin (see below) and promote neutrophil–platelet–endothelial cell interactions. It was recently demonstrated that platelets are a major source of soluble CD40 ligand (also known as CD154) [140]. CD40 ligand belongs to the TNF superfamily of molecules and has multiple actions that may be of significance within the microcirculation in sepsis [140]. These actions include upregulation of cytokine chemokine expression on vascular smooth muscle cells and endothelial cells; increased expression of surface adhesion molecules on endothelial cells; and upregulation of TF synthesis on macrophages [141].

P-selectin expression on endothelium and platelets is mediated by thrombin. It is a major surface adhesin that promotes the initial rolling and tethering interactions between circulating granulocytes, monocytes, and lymphocytes to endothelial cells at sites of tissue injury [138,142]. P-selectin glycoprotein ligand-1 is expressed on neutrophils, monocytes and some lymphocytes, and specifically binds to P-selectin on endothelial and platelet surfaces [142]. Thrombin-induced P-selectin expression on the endothelium promotes white cell adherence and cellular activation within capillaries and postcapillary venules. Activated neutrophils, in turn, release elastase that destroys antithrombin and cleaves TFPI. The loss of antithrombin and TFPI further disrupts the endogenous control mechanisms for thrombin generation, leading to a potentially lethal state of systemic activation of coagulation and inflammation.

E-selectin also facilitates neutrophil–endothelial cell interactions in the tissues. The E-selectin binding between neutrophils and endothelial cells is attenuated *in vitro* by protein C [143]. It has been speculated that the deficiency in protein C that accompanies severe sepsis contributes to the observed exaggerated neutrophil-mediated endothelial injury [18,23,143]. By these mechanisms, thrombin generation itself is now recognized as a potent inducer of proinflammatory reactions within the microcirculation.

Thrombin-initiated endothelial IL-6 production stimulates TF expression and further perpetuates ongoing coagulation [18,136]. This positive feedback loop between clotting and inflammation terminates in disseminated intravascular coagulation DIC and septic shock (the 'vicious cycle' of clotting and inflammation sepsis). All of these actions promote neutrophil, lymphocyte, and platelet interactions with the capillary endothelium, and this results in diffuse endothelial injury, increased vascular permeability, and cellular apoptosis.

The realization that thrombin activation not only initiates fibrin deposition but also activates a proinflammatory reaction has prompted efforts to inhibit thrombin generation in patients with severe sepsis [10,30]. The hypothesis that has been generated indicates that a potent inhibitor of thrombin activation should both prevent intravascular fibrin deposition and microcirculatory failure and provide an anti-inflammatory message to attenuate the proinflammatory state that typifies septic shock. However, at least with respect to the protein C system, inhibition of thrombin would diminish APC generation and thus suppress the anti-inflammatory and profibrinolytic activities of APC. Indeed, in comparative baboon models, natural anticoagulants have worked effectively in prophylaxis against *E. coli* infusion, but a very potent and specific anticoagulant, namely active site blocked factor Xa, failed [144].

Conclusion

The coagulation system is integrally related to the innate immune response, and its activation and regulation is dependent on local and systemic immune responses. The simultaneous activation of clotting and the innate immune response is a phylogenetically ancient host response to tissue injury, and has become the primary survival strategy throughout the long history of vertebrate evolution. This close linkage between clotting and inflammation has proven to be a survival advantage in response to the vicissitudes of life on earth, in which multicellular animals must constantly compete with well equipped microbial pathogens.

The molecular events that control coagulation are increasingly understood and the genetic elements that regulate the clotting and immune systems are being defined by human genome studies. It is evident from detailed experimental study that the dysregulated coagulation system that typifies the pathophysiology of septic shock contributes to systemic inflammation and lethality in sepsis.

The results of the initial clinical trials with recombinant human APC verify that it is possible to reverse the pathologic events that follow the onset of human sepsis and significantly improve the outcome of critically ill patients. It is hoped that the knowledge gained in unraveling the pathophysiology of coagulation and inflammation will result in further refinements and improved therapies for patients with severe systemic injuries and septic shock.

Competing interests

None declared.

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