Inflammation, innate immunity and blood coagulation

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Keywords
Thrombosis, inflammation, sepsis, multiorgan failure

Summary
Inflammation drives arterial, venous and microvascular thrombosis. Chronic inflammation contributes to arterial thrombotic complications, whereas acute inflammation drives venous thrombosis and microvascular thrombosis. Mechanistically, inflammation modulates thrombotic responses by upregulating procoagulants, downregulating anticoagulants and suppressing fibrinolysis. The inflammatory response can also result in cell apoptosis or necrosis. Products released from the dead cells, particularly histones, propagate further inflammation, tissue death and organ failure. Inhibition of histone mediated cytotoxicity appears to be a new mechanism for protecting against this deadly cascade.

Schlüsselwörter
Thrombose, Entzündung, Sepsis, Multiorganversagen

Zusammenfassung
Entzündung fördert arterielle, venöse und mikrovaskuläre Thrombosen. Eine chronische Entzündung trägt zu arteriellen Thrombosekomplikationen bei, während ein akute Entzündung venöse und mikrovaskuläre Thrombosen fördert. Mechanistisch gesehen modu-

iere eine Entzündung die Thromboaseantwort durch Hochregulierung von Prokoagulanzien, Herunterregulierung von Antikoagulanzien und Unterdrückung der Fibrinolyse. Die Ent-

zündungsreaktion kann auch zur Apoptose oder Nekrose von Zellen führen. Von abge-

storbenen Zellen freigesetzte Produkte, vor allem Histone, fördern zusätzlich Entzündung, Gewebeverletzung und Organversagen. Eine Hemmung der histonvermittelten Zyto-

otoxizität stellt anscheinend einen neuen Schutzmechanismus gegen diese tödliche Kaskade dar.

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There are links between inflammation and blood coagulation.

On the arterial side, inflammation is clearly associated with the development of cardiovascular disease (6), but most of the studies are based on correlation rather than cause and effect. One very nice example of a cause and effect association indicating directly that inflammation contributes to cardiovascular disease was reported by Liu et al. (7) where the investigators demonstrated that IL-6 polymorphisms that increase IL-6 levels were associated with an increased risk for cardiovascular disease.

On the venous side, one would anticipate that inflammation would also contribute to thrombotic disease. However, chronic low level inflammation that is associated with arterial thrombosis did not appear to be associated with venous thrombotic disease (12).

On the other hand, acute inflammation does contribute to venous thrombosis and pulmonary embolism (11). The latter findings are consistent with known changes that occur following an acute inflammatory response (Fig. 1). Many of these changes are consistent with two of the three components of Virchow’s triad, specifically increases in blood coagulability and changes in the vessel wall.

In addition to the inflammatory cytokines like tumor necrosis factor and interleukin 1, infection can trigger the release of neutrophil extracellular traps (NETs). These nets consist of DNA and nuclear proteins. The nets function to trap and clear pathogens from the circulation, reviewed in (8). While this is a beneficial immune function of the NETs, it comes at a cost—the NETs lead to vascular injury in vitro and liver damage in vivo (3). How the NETs cause tissue injury remains largely unknown.

Surgery and trauma are among the most common risk factors associated with venous thrombosis (4). They share with acute inflammation an increase in cellular necrosis and other forms of cell death, raising the possibility that factors derived from the dying cells might contribute to the propagation of the thrombotic response. Consistent with this notion is the clinical observation that organ failure is often associated with a progressive, rapid onset of multiorgan failure. Trauma and severe sepsis are two common causes of multi-organ failure (2). Activated protein C (APC) has been shown to be effective in decreasing death from sepsis, most particularly in patients with multiple organ failure (1, 10). The basis for this observation is unclear mechanistically since APC’s known functions include cytoprotection, anti-inflammatory activity and vascular barrier function protection (9), all of which might be expected to be most beneficial early in the septic response.
This clinical observation led us to search for other factors that APC might modulate. We stimulated a mouse macrophage cell line with endotoxin and gamma interferon under the assumption that the “other” mediators might be released into the media and be proteolytic substrates for APC. Indeed, gel analysis revealed three major new protein bands when the media was incubated with APC (13). As expected, the media was toxic to endothelium before incubation with APC, but toxicity was lost following APC treatment, raising the possibility that proteolysis to generate the new proteins was responsible for endothelial cell protection. Sequence analysis of the new protein bands identified them as histones.

Purified histones, especially histone H3 and H4, were toxic to endothelium at modest concentrations. Proteolytic cleavage of the purified histones with APC did block the cytotoxicity, suggesting that histones might contribute to tissue injury.

Examination of plasma samples from septic mice, baboons and some patients revealed the presence of histones at levels comparable to those required for cytotoxicity. Infusion of histones into mice resulted in dose dependent death with tissue analysis showing pathology very similar to that of sepsis, including fibrin formation and platelet deposition in the lung, leukocyte sequestration in the lung and fibrin formation within the alveolar space (Fig. 2).

Together, these results established that histones are toxic to endothelium, that they are released in sepsis, and that they propagate a thrombotic/inflammatory response. The results, however, left open the question of whether extracellular histones are important mediators of sepsis. To address this question, we obtained an antibody to histone H4 that blocked this histone’s toxic effects. When mice were challenged with a normally lethal dose of endotoxin and given the anti-H4 mAb, the mice were protected from death (13) (Fig. 3).

These investigations began with asking whether APC might function by inactivat-
Inflammation, immunity, coagulation

Histone-induced ultrastructural changes in the lung: Histone infusion (a-c) leads to neutrophil (PMN) adherence to microvascular endothelium and infiltration (b and c versus a, control), vacuolization of epithelial and endothelial cells, microvascular ruptures (d) with bleeding into alveolar space (b and c; RBC, red blood cells) and macro- and microvascular (d-f) thrombi rich in platelets, fibrin and sometimes erythrocytes. Magnification bars: a and b, 25 μm; d and e, 100 μm; c and e, 5 μm.

Fig. 2

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Fig. 3

Ten mice in each group were treated with LPS (10 mg/kg body weight). Survival at seven days post challenge was compared between groups.

% Survival

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Conflict of interest
Charles Esmon is a consultant for Artisan Therapeutics and Cardiome Pharma Corp, and holds a license agreement with Baxter.

References