Review
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 anti-coagulants 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

Keywords
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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 multi-organ 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.

Hämostaseologie 1/2010
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-
ing an important mediator of sepsis. Indeed that seems to be the case since APC can cleave histones and prevent histone cytotoxicity. Whether this is a key mechanism responsible for APC protective function in sepsis is unclear.

However, when mice were challenged with histones at levels that were normally lethal, the mice were protected if exogenous APC was also present, indicating that APC can block histone mediated injury in vivo as well as in cell culture.

However, inactivation of the histones by APC is relatively slow, suggesting that other approaches to blocking the histone cytotoxicity, such as the antibody used in the present studies, might be more effective than APC.

Although these studies were focused on models of severe sepsis, the findings may have ramifications with respect to many disease processes, including thrombosis. Ischemia induced by thrombotic occlusion is likely to result in necrosis and/or apoptosis with the release of nuclear proteins including histones. Consistent with this hypothesis, nucleosome levels (composed of DNA and histone) are known to rise in diseases where cells are stressed including trauma, cancer, auto immune disease and stroke, all with thrombotic complications associated with disease progression (5). Whether inhibition of histone toxicity will impact thrombosis or overall clinical outcome in these diseases remains to be determined. Also unknown is whether post-translational modifications of the histones are important in this process and indeed precisely which cellular determinants (receptors) play a role in histone toxicity. Of interest, these studies suggest novel mechanisms to interrupt the organ failure cascade that leads to death in sepsis and trauma. They also provide some insights into why APC clinically is effective in treating patients with multiorgan failure.
Conflict of interest
Charles Esmon is a consultant for Artisan Therapeutics and Cardiome Pharma Corp, and holds a license agreement with Baxter.

References