Inflammation and Thrombosis – Brothers in Arms

Elena M Faioni1 and Marco Cattaneo2

1. Senior Researcher, Internal Medicine, Department of Medicine, Surgery and Dentistry, University of Milan;
2. Professor, Internal Medicine, University of Milan, and Director of Medicine III, San Paolo Hospital, Milan

Abstract
Several interactions occur between inflammation and thrombosis that become more apparent with ageing. It is a two-way process: inflammation promotes aggregation and coagulation, while activated platelets and coagulation factors or peptides promote inflammation by several mechanisms. Recent evidence points to the role played by platelets and possibly coagulation factors in the regulation of innate immunity. Physiological anticoagulant pathways also have anti-inflammatory properties. This strong interdependence has an evolutionary origin. Current viewpoints are that inflammation, and thus coagulation, have a role in ageing. “Inflamm-ageing” is a neologism that denotes a low-grade inflammation that accompanies healthy ageing, but can evolve towards excessive inflammation and clotting. It can thus be associated with thrombosis and early death. These fascinating pathophysiological discoveries provide novel targets for the development of new, potentially efficacious antithrombotic drugs.

Keywords
Inflammation, thrombosis, ageing, immunity, platelets, anticoagulant pathways

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Correspondence: Elena M Faioni, University of Milan, via A Di Rudini, 8, 20142 Milan, Italy. E: elena.faioni@unimi.it

In the field of thrombosis a distinction has traditionally been maintained between venous and arterial thrombosis in terms of both the composition of the thrombus (fibrin- and cell-rich red thrombus in the veins versus platelet-rich white thrombus in the arteries) and risk factors. Venous thrombosis has been associated with hypercoagulability and/or reduced blood flow, whereas arterial thrombosis has been linked to atherosclerosis. However, more recently clinical epidemiology has suggested that risk factors for venous and arterial thrombosis in part overlap.1,2 This rather subversive observation is apparently without an explanation and seems, at first glance, to contradict everything that has been accepted about thrombosis for decades.

Chronic inflammation is increasingly recognised as a fundamental pathogenetic mechanism in a large number of diseases and conditions associated with an increased prevalence of thrombosis. Table 1. C-reactive protein, an established marker of inflammation, is known to be associated with arterial thrombosis3 and was recently shown to be independently associated with venous thromboembolism.4 Inflammation does not occur alone, or so practitioners like to believe: it is usually associated with infection or some other kind of toxicity, such as persistent hyperglycaemia or immune response, whether physiological or pathological, so it can be difficult to separate the effect of toxicity from that of inflammation.

Recently, experimental evidence has accumulated in terms of the effect of chronic inflammation, whatever its origin, on haemostatic response and endothelial and immune function. The main results of this research point to the conclusion, at least provisionally, that at some point in chronic disease progression inflammation does occur alone and is sufficient by itself to provoke and maintain a prothrombotic condition. Moreover, inflammation and its regulation is emerging as a fundamental feature of ageing, giving rise to the new term ‘inflamm-ageing.’3 On the whole, the evidence points to the fact that haemostasis and inflammation are tightly linked and that platelets and coagulation factors participate in inflammation and immune responses. The resulting prothrombotic phenotype predisposes to both arterial and venous thrombosis.

Inflammation and Platelets
Platelets play a major role in haemostasis: they bind to the exposed subendothelium, even at high shear, expose procoagulant surfaces and secrete vasoactive proaggregants, procoagulant substances and growth factors. Platelets thus participate in amplification of the coagulant response and the formation of stable clots and vessel wall repair. Recent evidence points to platelets being inflammatory cells.5 On activation, platelets express new surface proteins, some of which are implicated in leukocyte recruitment and chemokine deposition. One example of such surface proteins is P-selectin. This serves as an anchor for inflammatory leukocytes, facilitating the delivery and immobilisation of platelet-derived chemokines on the activated endothelium.6,7 Another important platelet surface receptor is CD40L, a tumour necrosis factor-alpha (TNF-α)-related transmembrane receptor implicated in inflammation and innate immunity.8 Recent evidence suggests that CD40L is involved in enhancing the signals
required for adaptive immunity, for example antigen-specific antibody production and allograft rejection. In inflammatory bowel disease, CD40L-positive platelets have been shown to adhere to mucosal microvascular endothelium in vivo, where they trigger or amplify a pro-inflammatory response. Platelets also secrete soluble CD40L (sCD40L), which in sickle cell anemia is elevated in plasma and biologically active. It therefore contributes to the induction of B cells, tissue factor and inter-Cellular adhesion molecule 1 (ICAM-1), suggesting that sCD40L may add to the chronic inflammation and increased thrombotic activity known to occur in this disorder. sCD40L interacts with another protein, type I transmembrane receptor CD40, which is expressed on platelet activation but is also present on monocytes/macrophages and endothelial cells. The sCD40L–CD40 interaction in this context promotes monocyte adhesion to vascular endothelium, endothelial activation and expression of leukocyte adhesion molecules.

Platelets have been shown to activate dendritic cells through the expression of CD40L in both in vitro and in vivo mouse models. Thus, through the co-stimulatory activity of dendritic cells, platelets are able to regulate T- and B-cell responses. Although the clinical significance of these observations has not yet been determined, it is interesting to note that increased expression of platelet activation markers, including CD40L, can be detected in several diseases with T- and B-cell components. These include inflammatory bowel disease, atherosclerosis, diabetes and systemic lupus erythematosus.

An activating capability of oxidised low-density lipoprotein (LDL) cholesterol on platelets mediated by the platelet receptor CD36 has recently been described and the chronic inflammatory feature of atherosclerosis. This activation can be further augmented by endothelial-cell-derived microparticles, which also bind and activate platelets via CD36. Besides hyperlipidemia, two other mechanisms might link obesity or the metabolic syndrome to thrombosis. The first is mediated by leptin, a hormone that regulates energy intake and is structurally and functionally related to cytokines. Leptin can enhance platelet aggregation when present in high concentrations, such as those observed in obese individuals. A second mechanism is observed in central obesity and involves insulin resistance. Platelets physiologically express insulin receptors. Insulin decreases the sensitivity of circulating platelets to agonists and platelet deposition under high shear rate conditions. This may be why platelets from patients with diabetes or those who are obese are less sensitive to the antiaggregating effects of insulin.

Another pathway linking innate immunity, haemostasis and thrombosis is the activation of a complement by platelets or platelet microparticles, supposedly through the expression of the C1q receptor on the platelet surface and the expression of P-selectin. The complement-activated components C3a and C5a are potent pro-inflammatory agents. The terminal complement complex C5b-9 has platelet- and endothelial-activating properties at sublytic concentrations. The platelet chemokine platelet factor-4 (PF-4) and beta-thromboglobulins have an important, although diversified, role in innate immunity as well as an established and well-known role in haemostasis.
most abundant chemokines secreted by platelets and are the first chemokines to be purified. Their elevated concentration and ready availability on platelet activation are associated with their role in early host defence. Contrary to other, similar chemokines, PF-4 does not promote cell migration but rather firmly positions neutrophils at the vascular endothelium. It favours release of metalloproteinases from secondary neutrophil granules, thus predisposing to extravasation. Furthermore, PF-4 can induce oxygen radical formation in monocytes. Beta-thromboglobulins are in fact several proteins differing in their N-terminuses. They result from proteolytic processing of the precursor platelet basic protein. One beta-thromboglobulin, neutrophil-activating peptide 2, is a potent chemotactic for neutrophils and stimulates the release of lysosomal and secondary granules.

**Inflammation, Coagulation, Naturally Occurring Anticoagulants and the Endothelium**

For more than two decades experimental results obtained in vitro, in animal models and in humans have shown that inflammation favours a procoagulant phenotype and, reciprocally, that coagulation factors have pro-inflammatory activities. Physiologically, the endothelium is an anti-inflammatory anticoagulant surface. It expresses glycosaminoglycans that enhance antithrombin function and it expresses thrombomodulin, which binds thrombin and activates the protein C pathway with the aid of the endothelial protein C receptor. The endothelium also synthesizes the tissue factor pathway inhibitor, which binds and inhibits the tissue factor-activated factor VII-activated factor X complex. Cytokines derived from activated leukocytes during inflammation downregulate the endothelial receptors and upregulate leukocyte adhesion molecules, while proteases from leukocytes cleave thrombomodulin and glycosaminoglycans from the endothelial surface. The net result is that the endothelium is tipped towards being a procoagulant, pro-inflammatory surface. At the same time, cytokines such as TNF-α and interleukin-1 (IL-1) induce tissue factor expression on monocytes and the endothelium, favouring unopposed thrombin formation. Thrombin in turn has several pro-inflammatory activities. It induces the expression of P-selectin and the secretion of platelet-activating factor by the endothelium. It is chemotactic for leukocytes. It also stimulates the production of monocyte chemotactic protein-1, IL-6 and IL-8. The inflamed endothelium loses its barrier function so leukocytes can extravasate in the extraluminal space. This is an initial step that occurs in chronic inflammatory diseases of vessel walls, such as atherosclerosis or vasculitis. In vasculitis, immune complexes that bind to the endothelium and trigger an upregulation of leukocyte adhesion molecules may be a source of arterial injury, initiating endothelial inflammation and atherosclerosis.

Downregulation of thrombomodulin and endothelial protein C receptor was reported in the mucosal microvessels of patients suffering from inflammatory bowel disease. It might contribute to ischaemia and necrosis of the intestinal mucosa. Patients with inflammatory bowel disease also develop thrombosis in vessels that are far from the gut. They have a more than three-fold higher risk of venous thrombosis than the control population or patients with other chronic bowel diseases, such as coeliac disease. The proposed mechanism is that tissue-factor-carrying microparticles or cells generated by inflammatory stimuli in the intestinal circulation eventually reach the systemic circulation and are the trigger for thrombosis.

Another important effector of inflammation is tissue hypoxia. Besides exerting a proangiogenic effect through upregulation of vascular endothelial growth factor, hypoxia determines a complex procoagulant response mediated by several cellular mechanisms. Oxidative stress, mediated by leukocytes and bacteria, also shifts the haemostatic balance. One specific and well-described mechanism, for example, is the oxidation of methionine 388 in thrombomodulin. This is known to slow the rate at which the thrombomodulin-thrombin complex activates protein C. It has been shown to be a potential mechanism of hypercoagulability in patients with diabetes and in those who smoke.

**Haemostasis and Immunity – An Evolutionary Perspective**

That haemostasis, inflammation and innate immunity are tightly linked is not so surprising. These defense mechanisms have developed together and were a single entity before vertebrates evolved. In the horseshoe crab, an invertebrate model for the study of the evolution of coagulation, external ‘agents’ disrupting its integrity activate a cell, called the amebocyte, which functions as both a platelet and a macrophage. The amebocyte degranulates on activation, releasing proteins that react to form an insoluble clot. At this point it releases a substance with antibacterial properties – a vestigial form of innate immunity. The result is the ‘clotting out’ and destruction of the disturbing agent. Vertebrates have evolved separate clotting and inflammatory immune systems that can co-operate and interact. Most of the inflammatory signals responsible for immune activation will also precipitate procoagulant signals.

Conversely, the coagulation system rapidly feeds back and upregulates the innate immune response. Anticoagulants possess several anti-inflammatory properties, which have been exploited therapeutically in settings such as severe sepsis. Their anti-inflammatory properties are independent of their antithrombotic action, as shown in the case of protein C by results obtained in animal sepsis models after separating the anticoagulant from the anti-inflammatory function. The interaction between inflammation, haemostasis and immunity is schematically represented in Figure 1.

**Inflammation, Ageing and Thrombosis**

In recent years it has become clear that ageing is associated with a low-grade persistent inflammatory response that has been termed inflamm-ageing. Coagulation factors and pro-inflammatory cytokines...
are increased in ageing individuals. Memory T-cell clones also
are and directed toward a few epitopes of common viruses, such as
cytomegalovirus and Epstein-Barr virus.14,15 There seems to be
a quantitative gradient in high levels of IL-6 (a pro-inflamm-atory cytokine often used as a measure of inflammation), for
example, are associated with an increased risk of frailty in the elderly.16,17
In healthy centenerians this low-grade inflammatory response is
associated with anti-inflamm-ageing, i.e. with biological mechanisms
that control inflammation.18,19 The natural conclusion from these
experimental observations is that excess inflammation is a noxious
impediment to healthy ageing. It is not currently known whether
this low-grade chronic inflammation and coagulation activation is
directly linked to the increasing incidence of thrombosis, both arterial
and venous, with age (the risk doubles for every decade after 40
years of age).20 It is likely, however, that in ageing – as in the chronic
diseases mentioned – persistent inflammation is a predisposing
factor for thrombosis. It is also plausible that other intervening
precipitating factors common in the elderly population, such as
cardiovascular disease, cancer and metabolic derangement, trigger
the thrombotic events.

Comments and Perspectives
Anti-inflammatory agents have traditionally been commonly used in
antithrombotic therapy: both aspirin and heparins possess
anti-inflammatory activity.21,22 However, aspirin, is often used at
antiplaсти dosages that do not elicit an anti-inflammatory effect (i.e. do
not inhibit cyclo-oxygenase-2 [COX-2]).23 Added to this, the
anti-inflammatory properties of heparin are exploited mostly in
the acute phase of thrombosis, since patients are soon placed on oral
anticoagulants. Anti-inflammatory drugs, in association with
antithrombotics, have the potential to interrupt the complex interactions
shown in Figure 1. Moreover, as mechanisms linking inflammation,
coagulation and immunity are unravelled, new therapeutic targets are
evening. Little is known about the complex pro-inflammatory/anti-
inflammatory balances that exist in chronic inflammation. The
importance of an individual’s genetic background in terms of
inflammatory and immune responses is just now beginning to be
appreciated. Research in this area is moving forward fast. It is also
focusing on the important role of neutrophils in thrombotic events,
especially in the arteries. New therapeutic strategies will hopefully be
born from the results of this exciting ongoing research.