Editorial
Should cardiologists forget about platelets and take an interest in blood leukocytes?

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When the cardiologists discovered platelets and thrombosis

Coronary thrombosis is currently accepted as the factor which precipitates acute myocardial infarction[1]. Until the advent of angiography there were controversies over the causal role of thrombosis. In the last 20 years, however, the successful use of thrombolytic therapy in acute ischemic syndromes[2,3] and the consistent positive results shown by aspirin and other antiplatelet drugs[4] convinced almost all cardiologists on the critical role of coronary thrombosis in acute ischemic syndromes and of platelet activation in the early phases of thrombus formation and growth. After plaque rupture, exposed collagen from the vessel wall, in addition to other mediators, induces platelet aggregation. Platelet-released substances, such as adenosine diphosphate and thromboxane A_2, stimulate adjacent platelets, further enhancing the process of platelet activation. Platelet membrane glycoproteins appear to play a role in the events of platelet adherence and aggregation and involve specific plasma adhesive proteins, such as von Willebrand factor and fibrinogen[5,6].

Blood leukocytes present on stage

More recently blood leukocytes too have been suggested to be involved in atherogenesis, thrombus formation and their regulation[7]. Epidemiological studies have shown evidence of a significant association between leukocyte count and incidence of coronary heart disease[8,9]. On one side monocytes/macrophages express tissue factor when stimulated by endotoxin or other inflammatory agents, thus contributing to blood clotting initiation[10], on the other side, macrophages, T lymphocytes and mast cells, infiltrating coronary lesions, may help to bring about plaque instability[7].

Additionally, polymorphonuclear (PMN) leukocyte-released substances, such as cathepsin G and elastase, may activate platelets[11,12] and degrade endothelial barrier function[13]. While these experimental data might suggest a no more unique role of platelets as a "thrombogenic cell", some recent studies appear to place again platelets in the pathophysiological center of thrombus formation and growth.

A new thrombogenic role of platelets

Indeed, activated platelets not only facilitate further platelet accumulation and fibrin deposition at the site of vascular injury – as it was clearly established in the past –, but may recruit PMN and mononuclear leukocytes into actively forming thrombus[14,15] by expressing P-selectin, a receptor mediating platelet-leukocyte binding[16-18] which has been shown to induce tissue factor in monocytes[19]. Moreover activated platelets release chemokines, which may undergo proteolytic cleavage to form neutrophil activating peptide-2[20,21].

The interaction between platelets and leukocytes is part of a larger chapter – that of the role of inflammation in acute coronary syndromes[22] – to which Italian investigators have actively contributed[23-26].

As already mentioned, platelets activated at the site of vascular damage may sub-

stitute endothelial cells in recruitment and migration of PMN leukocytes through damaged vessel wall[27]. Leukocytes accumulated in a platelet thrombus can contribute to further platelet activation and deposition. These events on one hand may facilitate the maintenance of the vascular and tissue integrity, on the other may play a pathogenetic role in inflammatory and thrombotic disease[28].

Circulating platelet-PMN complexes are formed by activated PMN with marked adhesion molecule profile and a great capacity for phagocytosis and toxic oxygen metabolite production[29]. Platelet contribution to the inflammatory response of the vessel wall is not limited to PMN activation, but also involves interaction with mononuclear cells, e.g. platelet arachidonate metabolites and P-selectin both stimulate tissue factor generation by monocytes[19]. Activated platelets are also able to bind to circulating lymphocytes and to mediate rolling in endothelial venules through exposed P-selectin[30]. More recently it has been shown that the administration of activated platelets into the systemic circulation of L-selectin-knock-out mice restores lymphocyte trafficking to peripheral lymph nodes and reconstitutes T cell-mediated immunity in response to a cutaneous antigen[31]. These data suggest that platelets may also play a pivotal role in lymphocyte recruitment.

A platelet machinery to catch flowing leukocytes

Several experimental studies indicate that platelets either adherent to a surface, or activated in suspension express a complete machinery to catch flowing leukocytes[28]; it includes:

- P-selectin, a membrane glycoprotein stored in the α-granules of platelets and exposed on the external surface after cell activation, which is responsible for the first reversible contact between platelets and leukocytes[16,17,31];
- one or more signaling molecules that are relevant for the activation of Mac-1, a leukocyte β_2 integrin[27,31-33];
- the counter receptors of the β_2 integrin on platelets[33-35].

Interestingly enough, a platelet receptor for leukocyte β_2 integrin is fibrinogen, bound to activated platelets through the exposed GP IIb/IIIa[34-36].

Based on experimental data, the hypothesis has been made that P-selectin allows activated platelets to stimulate tyrosine kinase-dependent adhesive properties of Mac-1. The binding of activated platelets to PMN triggers in the latter cells rapid tyrosine phosphorylation of a protein of about 110 kD, provisionally called P110[33,37].

New pharmacological avenues

These data may open new perspectives for prevention of cardiovascular events after acute coronary syndromes, in the same moment when a large clinical trial such as the SYMPHONY study reported deceiving results on a new antiplatelet approach. Indeed, the frequency of the primary endpoints (all-cause mortality, nonfatal myocardial infarction, or severe recurrent ischemia) in patients with acute coronary syndromes did not differ between aspirin and a new oral GP IIb/IIIa inhibitor (sibrafiban)[38].

Drugs interfering with platelet-leukocyte interaction, thus blocking both platelet and leukocyte contribution to thrombus formation, might prove to be clinically effective more than drugs only affecting either cell type[39]. Although platelet GP IIb/IIIa might be indirectly involved in platelet-leukocyte interaction mediated by fibrinogen, compounds directly inhibiting the function of P-selectin, β_2 integrin or their respective counter-receptors should be identified and developed for experimental and clinical testing.

A recent study in patients undergoing coronary angioplasty[40] has demonstrated that, although abciximab (an anti-GP IIb/IIIa chimeric monoclonal antibody) treatment decreases the percentage of circulating leukocyte-platelet aggregates, it promotes P-selectin-mediated leukocyte-platelet interaction on a collagen-von Willebrand factor surface. On the other hand platelet-monocyte interaction decreased after abciximab treatment in patients with acute myocardial infarction, mainly due to a reduction in platelet mass attached to monocytes, as it did not affect the percentage of monocytes with adherent platelets[41].

Following the original observation of Palabrica et al.[42] that platelet P-selectin-dependent leukocyte accumulation in an experimental thrombosis model in the baboon promotes fibrin deposition, it has been shown that P-selectin blockade by specific monoclonal antibodies[43] or with a soluble recombinant form of P-selectin glycoprotein ligand-1[44] – the P-selectin receptor on leukocytes – accelerates the pharmacological lysis of arterial thrombi and prevents reocclusion in animal models. P-selectin blockade also reduces PMN adhesion to platelets at the site of a deep injury in the carotid artery of pigs[45] and reduces cyclic occlusion in a canine model of recurrent arterial thrombosis[46].

Compounds interfering with the intracellular signaling mechanisms, including the control of P110 protein, might be of some pharmacological and therapeutic relevance. In this context, it may be of interest to mention that genistein, piceatannol and other relatively specific inhibitors of leukocyte tyrosine kinases have been found in natural-derived nutrients or in wine[47]. And wine is presently considered as a dietary means for preventing ischemic heart disease[48]. Our group has also shown that platelet-PMN adhesion and Mac-1 expression in vitro is prevented by trans-resveratrol, a polyphenolic compound contained in red wine[49].

Platelet-leukocyte interaction: pathophysiological relevance

The pathophysiological relevance of leukocytes de-

posited on platelets activated within the circulating blood and/or adhering at the site of vascular damage is still largely unknown. However the presence of platelet-PMN conjugates has been reported in peripheral blood of patients with unstable angina[50], suggesting that this cell-cell interaction triggers PMN activation in this clinical condition[51], a finding originally reported by Mazzone et al.[24]. On the other hand, Mickelson et al.[52] found that formation of platelet-PMN conjugates following coronary angioplasty was a predictive index of acute reocclusion.

In patients with acute myocardial infarction increased platelet-monocyte interaction and up-regulation of Mac-1 on monocytes have recently been reported[53].

The intriguing possibility is emerging that platelets activated at the site of an unstable atherosclerotic plaque are unable by themselves to produce a full vascular occlusion, but might be the initial trigger of a localized leukocyte-dependent inflammatory response.

Platelet-PMN interaction contributes to the increased production of vasoactive metabolites, such as thromboxane A_2 and leukotriene C_4[54], may damage endothelial cells and impair endothelium-dependent fibrinolytic response[13].

Acute coronary syndromes are associated with inflammatory reactions, as indicated by the elevated levels of circulating C-reactive protein[25]. In addition, high fibrinogen levels and elevated leukocyte count are predictive of the risk of myocardial infarction[9,55,56]. Platelet-PMN interaction as well as the consequent PMN activation may be an essential part of this inflammatory response and might constitute a new parameter to predict the risk and to monitor the severity of ischemic disease.

To ape Shakespeare, if the platelet is a “shrew”, its taming appears still far to be fully achieved.

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