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# Platelets: new players in the mucosal scenario of inflammatory bowel disease

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*Abstract.* – **Besides their classical haemostatic task, platelets are now recognized as novel cells deeply involved in inflammation. Compelling evidence support their role as active player in mucosal tissue injury that occurs in Crohn's disease and ulcerative colitis. In both forms of inflammatory bowel disease platelets contribute to microvascular endothelial activation and recruitment of inflammatory cells, thus fostering and amplifying mucosal inflammation.**

*Key Words:*

*Platelets, Endothelium.*

## Introduction

Both Crohn's disease (CD) and ulcerative colitis (UC) are characteristically chronic diseases resulting from the combined contribution of environmental, genetic, bacterial and immune factors[1]. The chronicity of symptoms is due to continuous tissue injury caused by unrelenting inflammation. Mounting evidence suggests that gut tissue injury results not only from an abnormal immune response, as traditionally acknowledged, but also the activation of other cellular systems[2]. The gut is composed of several cell types, and a complex interplay of immune-nonimmune cell interactions regulates intestinal immunity and inflammation[4]. That nonimmune cells are as important as immune cells in chronic inflammation, a concept barely explored in inflammatory bowel disease (IBD), is established in other conditions. In rheumatoid arthritis the dominant role of T-cells in the beginning of disease is succeeded, in the chronic phase, by a self-perpetuating pathway of inflammation mediated by activated synoviocytes[3]. In celiac disease the role of fibroblast- and endothelial cell-derived tissue transglutaminases for gliadin recognition by and activation of mucosal T-cells is central to pathogenesis[4]. In atherosclerosis, another form of chronic inflammatory disease, the persistent activation of endothelial cells by CD40L+ platelets and CD40L+ T-cells plays a central role in its pathogenesis[5]. Platelets, an other type of nonimmune cells, have been very recently recognized to actively participate to chronic IBD[6]. In the literature it has been always been strong the evidence for vascular dysfunction in IBD, such as hypercoagulability and thrombosis It is well established that a hypercoagulable state exists in IBD at both the systemic and intestinal level as demonstrated by several clinical observations[6]. There is a high incidence of peripheral vein thrombosis (up to 41% in post-mortem studies), representing an important cause of morbidity, frequently associated with smoking and oral contraceptive use, known thrombotic risk factors[6]. Increased serum levels of von Willebrand factor, a marker of endothelial damage, suggest the existence of diffuse endothelial injury in IBD[7], and inherited disorders of coagulation, such as hemophilia and von Willebrand's disease, are protective against IBD[8]. Moreover, the apparent therapeutic benefit of heparin provides further support that thromboembolic phenomena are linked to IBD pathogenesis[9]. In CD mucosa multifocal mesenteric microinfarctions and microvascular thromboses are frequently found, often intimately associated with granulomatous inflammation[10]. Such vascular damage has been proposed as an early event in IBD pathogenesis, leading to platelet acti-

vation and aggregation followed by accumulation of macrophages around the mucosal blood vessels and eventually tissue injury[7,11]. All these "coagulation" events happening in IBD have been enriched by a novel role of platelets as active participants in intestinal inflammation.

Aim of this review is to elucidate the recent data supporting the active role of platelets in participating in mucosal inflammation in both forms of IBD.

### Platelets: an Abundant Source of Potent Inflammatory Mediators

Platelets are unique anucleate mammalian blood cells that are critically involved in hemostasis. Beyond this classical task, they display specialized molecular repertoires that have evolved to accomplish crucial functions in immunity and inflammation. Indeed platelets are active participants of the multicomponent system of inflammation and tissue repair that follow endothelial activation and injury[12,13]. Similarly to mast cells, platelets store considerable amounts of preformed, biologically active substances which induce or regulate inflammatory reactions[12,13]. Among such substances are histamine, $PGE_2$, $PGD_2$, PDGF, and serotonin which alter vascular permeability and mediate vasodilation or vasocostriction[14,15]. Other substances cause neutrophils activation and degranulation, like adenine nucleotides, PDGF, and platelet factor 4, while others cause fibroblast proliferation and wound repair like TGF-β. Notably, platelets also release major chemoattractant molecules, including the chemokines RANTES and MIP-1α, as well as PAF and the leukotriene 12-HETE. Most of these mediators are actively released by activated platelets at sites of inflammation and tissue injury thus promoting a further recruitment of inflammatory cells. In addition to these classical pro-inflammatory molecules, activated platelets secrete soluble sCD40L, a protein homologous to members of the tumor necrosis factor (TNF) family, which engages CD40 on the surface of most immune cells, including T- and B-cells, monocytes and macrophages, as well as nonimmune cells such as mesenchymal and endothelial cells[16,18]. Notably, the expression and release of CD40L by activated platelets not only promotes immune activation and inflammation, but also contributes to coagulation by inducing tissue factor production by endothelial cells and monocytes[19,20]. Finally, activated platelets are able to produce IL-1β and IL-7 which, through their numerous and potent biological properties, further widen platelets' range of pro-inflammatory and immunoregulatory functions[21,22].

### PLT-endothelial Interaction: a Major Component of the Inflammatory Response in Autoimmune, Allergic and Vascular Diseases

The large variety of mediators released by platelets allows them to interact with a number of immune and nonimmune cells. For instance, activated platelets signal human monocytes to produce chemokines such as IL-8 and MCP-1[22]. However, the most biologically relevant interaction is with the vascular endothelium[23,24]. As a result of this interaction endothelial cells become activated and produce cytokines, chemokines and upregulate cell adhesion molecules (CAM), all of which contribute to amplify and sustain inflammation. IL-6 and GM-CSF are released by platelet-activated human umbilical vascular endothelial cells (HUVEC)[25], which also produce chemokines mediating neutrophil and monocyte recruitment, such as IL-8 and MCP-1[26,27]. Platelets also enhance expression of ICAM-1 and E-selectin by HUVEC[27]. The platelet-induced endothelium activation is a contact-dependent process where the expression of CD40L by activated platelets allows binding to and stimulation of CD40-bearing endothelial cells[27-29]. The importance of the CD40/CD40L system in platelet-endothelial cell interaction is demonstrated by the inhibition of chemokine production and downregulation of CAM by CD40L blocking antibodies[27-30].

Activation of platelets is a common feature in several chronic clinical conditions of autoimmune, allergic and vascular origin. In rheumatoid arthritis platelets exist in an activated state in the synovial fluid of affected joints, while in systemic lupus erythematosus circulating platelets display significantly enhanced activation markers[31,32]. Platelet-derived soluble CD40L apparently mediates the febrile response to transfusion[33], and may play a pathogenic role in acute coronary syndromes[29].

### Evidence of Platelet Dysfunction in IBD

Platelets play a major role in vascular complications of IBD patients. During active disease platelets are markedly increased in number ("reactive thrombocytosis") and activation state, displaying enhanced aggregation in *vivo* and *in vitro*. Enhanced aggregation is likely due to the fact that in IBD platelets circulate in an activated state, as shown by enhanced P-selectin and GP53 expression, and increased production of b-thromboglobulin, platelet factor 4, and thromboxane[34-36] $B_2$. Markers of platelet activation are higher in the microcirculation of CD patients compared to the systemic circulation[37]. Furthermore, platelets express enhanced IL-1 and IL-8 receptors in IBD compared to healthy subjects, receptor density being inversely correlated to anti-inflammatory therapy[38]. Finally, aminosalicylates are know to reduce platelets activity, and this action could explain, at least in part, their therapeutic effect in IBD patients[39].

The most recent confirmation of a heightened platelet activation state in IBD is the detection of surface CD40 ligand (CD40L), an activation marker that allows platelets to interact with a broad variety of immune and nonimmune cells (17). Compared to healthy controls, circulating platelets in both CD and UC patients have significantly greater expression of this potent immunoregulatory and pro-inflammatory molecule than normal platelets, and this difference persists even after *in vitro* thrombin stimulation[40]. These CD40L-positive platelets are essentially the only source of the increased plasma levels of the soluble form of CD40L (sCD40L) in IBD, which result from the enzymatic release of this molecule from the surface of activated platelets into the peripheral and mucosal circulation[41].

### Platelet Participation to Intestinal Chronic Inflammation

The initial suggestion that platelets display an activation state at mucosal level was reported in histopathological studies revealing the presence of mucosal capillary thrombi in rectal biopsies of patients with IBD[42]. Intravascular microthrombi are frequently observed in CD and UC mucosa, even though their presence is unrelated to the severity of inflammation, and they are consistently absent in the mucosa of normal subjects[42]. Furthermore, the finding of an increased expression of the pro-coagulant molecule tissue factor, which closely correlates with the degree of thrombosis in the mucosal microvasculature of CD patients, enriched the bridge between coagulation and platelet dysfunction in IBD[43]. In fact, one of the earliest abnormalities in CD mucosa is the presence of platelet thrombi cross-linked with fibrin in the mucosal microvasculature[44]. This feature, however, is not specific of CD as can be found in other idiopathic inflammatory bowel disorders[43]. In reality, the intimate adherence of platelets to the endothelium is a general phenomenon characteristic of the early manifestations of regional immune reactivity[45], and persists throughout the course of several inflammatory conditions, including IBD.

Critical informations suggesting that the activation of platelets occurs in the inflamed intestinal circulation in IBD has been described by Collins et al, where the authors describes increased platelet aggregates in the mesenteric blood of CD patients[46]. The same event was recently reproduced *in vitro* using platelets co-cultured with human intestinal microvascular endothelial cells (HIMEC). HIMEC pre-treated with IL-1β to mimic IBD endothelium can activate platelets through simple physical contact, as evidenced by a upregulation of P-selectin and CD40L expression on the platelet surface[41].

Additional evidence of their involvement in mucosal inflammation is the recent demonstration that IBD platelets express high levels of surface CD40L, creating a physical and biological bridge that allows interaction with and activation of HIMEC. This series of events actually occurs, as CD40L-positive platelets in IBD have been detected *in vivo* adhering to mucosal microvascular endothelium where they trigger or amplify a pro-inflammatory response[40]. The *in vitro* counterpart for this finding is the upregulation of two crucial adhesion molecules involved in leukocytes adhesion, vascular adhesion molecule (VCAM)-1 and intercellular cell adhesion molecule (ICAM)-1, by activated IBD platelets through the CD40-dependent pathway. Through this same pathway IBD platelets also stimulate HIMEC to produce IL-8, the major neutrophil chemoattractant, setting in motion HIMEC's signaling machinery along the MAP-kinase cascade, and pro-

moting a marked phosphorylation of p38. It is worthy of note that platelets can activate various cells not only through contact with membrane-bound CD40L, but also through the release of its soluble form, representing still another paracrine mechanism of inflammation. For instance, sCD40L can activate intestinal resident cells such as fibroblasts and HIMEC, inducing them to secrete chemokines, up-regulate VCAM-1 and ICAM-1, and enhance T-cell adhesion to endothelium and subsequent transmigration into the interstitium[47].

In addition to IL-8, IBD platelets release, upon contact with HIMEC, profuse amounts of biologically active RANTES[36], a chemokine critical for recruitment of monocytes and memory T-cells and strongly expressed by endothelial cells surrounding granulomas. HIMEC avidly immobilize and retain on their surface the platelet-derived RANTES which can thus mediate adhesion of more T-cells to HIMEC. This sequence of events probably translates the unfolding of an *in vivo* inflammatory cycle, where platelet-triggered, chemokine-mediated leukocyte adhesion to endothelium occurs that subsequently results in leukocyte transmigration into the interstitium to create a focus of inflammation. This cycle links platelet activation and T-cell recruitment, and implicates platelets in cell-mediated immune phenomena in gut inflammation[25]. Another functional link between platelets and leukocytes has been recently postulated. T-cells adhering to an inflamed microvascular bed may create an effective platform onto which platelets bind and thus further interact with the endothelium itself[49].

An other contribution of activated platelets to IBD-associated mucosal damage is suggested by the intriguing observation that UC platelets enhance the production of reactive oxygen species by polymorphonuclear leukocytes[50]. This may contribute not only to the high levels of reactive oxygen species at mucosal level, but also to mediation of mucosal injury in this condition[51].

A recent report has described a further contribution of platelets to the recruitment of T-cells at sites of tissue injury and inflammation. Platelets express constitutive and functional CD40, that allows activated CD40L positive T-cells to bind and to trigger platelet activation. These events lead to up-regulation of P-selectin and granular RANTES release, that at endothelial sites is a potent recruiter of memory T-cells[52].

## Conclusions

The rapidly expanding information on the role of platelets as mediators of inflammation, combined with the previously acquired but still evolving knowledge on the involvement of platelets in IBD[53,54], suggest a novel role of this cell type in mucosal inflammation. Indeed, because activated platelets are previously unsuspected but active coconspirators of inflammation and tissue injury in a wide range of inflammatory conditions, they may be close to the degree of pathogenic relevance attributed to classical immune cells. As a consequence, platelets are well on the way to acquire a higher degree of relevance in the complex mosaic of IBD pathogenesis[55]. Because both their number and state of activation are markedly increased during the active and even inactive stage of CD or UC, their presence represents a significant risk factor for amplification of gut inflammation, and makes them a rational target for specific therapeutic intervention.

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