

## CLINICAL REVIEWS

# Platelets in Inflammatory Bowel Disease: Clinical, Pathogenic, and Therapeutic Implications

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Both Crohn's disease (CD) and ulcerative colitis (UC) are associated with abnormalities of platelet number and function. In the peripheral circulation the state of platelet activation is typically increased, and inflammatory bowel disease (IBD)-involved mucosa frequently contains platelet aggregates within mucosal microthrombi. The relevance of platelet dysfunction to IBD pathogenesis is still unclear, but there is solid evidence demonstrating that platelets, in addition to their traditional role in hemostasis, can also function as potent proinflammatory cells. Upon activation, platelets secrete a large number of biologically active molecules able to induce or amplify an inflammatory process through many of the same cellular and molecular pathways conventionally utilized by immune cells mediating IBD. The aim of this article is to review data on the existence of platelet dysfunction in IBD, substantiate platelets' inflammatory potential, discuss the implications of abnormal platelet activity for chronic intestinal inflammation, and consider the potential benefits of platelet modulation for treatment of IBD.

## INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of inflammatory bowel disease (IBD), appear to result from the combined interaction of environmental, genetic, immune, and microbial factors (1). Mounting evidence suggests that tissue injury in IBD is not exclusively the outcome of a dysregulated immune response, as conventionally accepted, but also involves the active participation of other cellular systems. The gut is composed of multiple cell types, and a complex interplay of immune–nonimmune cell interactions regulates intestinal immunity and inflammation (2). Platelets are anuclear nonimmune cells derived from the cytoplasm of bone marrow megakaryocytes, and they play a key function in blood hemostasis. To accomplish this highly specialized task, platelets continuously circulate in the blood stream surveying vessel integrity and discriminating between intact and injured endothelium. During the last decade it has become evident that, in addition to their primary hemostatic function, platelets also play an active role in a variety of inflammatory processes (3, 4). In particular, the contribution of platelets to cardiovascular disease-associated inflammation is now recognized as a major pathogenic component (5–7). In the following sections, we will show that platelets are dynamic participants of the multifaceted process of chronic intestinal inflammation, and represent an important though previously underestimated component of IBD pathogenesis.

## PLATELET DYSFUNCTION IN IBD

The first evidence of a platelet abnormality in IBD was reported in 1968, with the description of an increased platelet

count in patients having an exacerbation of clinical activity (8). Since then, it became established that "reactive thrombocytosis" (defined as a platelet count  $>450 \times 10^9/L$ ) is a common feature during the active phase of IBD (9). The high platelet number correlates well with disease severity and serum orosomucoid concentration, a marker of systemic inflammation, and, interestingly, may persist even after bowel resection (10–12). Based on these observations, platelet count has been proposed as a simple method to distinguish IBD from infectious diarrhea (13). The reason for the increased number of platelets in the circulation of IBD patients is not well understood, but it is usually considered to be a nonspecific response to inflammation similar to what occurs in other chronic inflammatory conditions, like rheumatoid arthritis or systemic lupus erythematosus. It has also been proposed that the thrombocytosis of CD and UC could reflect a disturbance of thrombopoiesis, as suggested by the increased plasma levels of thrombopoietin and interleukin (IL)-6, two critical factors involved in megakaryocytic maturation (14). On the other hand, data regarding IBD platelet survival time are contradictory, some studies showing a normal and others showing a reduced platelet lifespan compared to platelets from healthy subjects (9, 15).

Another interesting aspect characterizing platelet dysfunction in IBD is their small mean corpuscular volume in the circulation of CD and UC subjects (16). Mean platelet volume has also been proposed as a potential marker of clinical disease activity, being inversely proportional to the levels of classical inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate. The cause of the reduced platelet volume in clinically active IBD is unknown, but it

may be a direct consequence of the thrombopoiesis disturbance often observed in the early stages of systemic inflammatory processes (17). Despite a reduced platelet volume, platelets in IBD have an augmented granular content, as indicated by studies performed with a computerized analysis of platelet density distribution (16).

Although abnormalities in platelet number, size, and density have been suggested as indicators of disease activity, the importance of platelets is also due to the substantially increased incidence of thromboembolic phenomena in both forms of IBD (18). *In vitro* studies have demonstrated that spontaneous platelet aggregation is present in more than 30% of IBD patients compared to none of controls, and independent of disease severity (15). An intriguing aspect of this complication is that it may not simply represent a consequence of chronic inflammation, but a characteristic of IBD, since platelet aggregates are found *in vivo* circulating in IBD patients but not patients with other types of chronic inflammation (19). Finally, compared to healthy control platelets, IBD platelets are more sensitive to activation induced by a variety of proaggregating substances, including adenosine diphosphate, collagen, ristocetin, and arachidonic acid (15, 20).

A significant advance in the field of platelet abnormalities in IBD was achieved with the findings of Collins *et al.* (19). Using flow cytometric analysis, these authors showed that in IBD platelets circulate in a highly activated state, demonstrated by the expression of surface activation markers such as P-selectin and GP53, and serum measurement of the platelet activation marker  $\beta$ -thromboglobulin ( $\beta$ -TG) (19). As for some other above-mentioned parameters, the increased platelet activation state was independent of clinical activity, perhaps suggesting that chronicity of the disease process could lead to enhanced platelet activation even if the disease is clinically silent. A complementary observation, likely to be relevant to disease pathogenesis, is that in CD the enhanced platelet P-selectin expression is found in mesenteric compared to peripheral venous blood, probably indicating that platelet activation occurs in the intestinal microcirculation (21, 22).

Corroborating the enhanced activation state of platelets in IBD are additional reports showing elevation of various products that are exclusively released by activated platelets, such as platelet factor 4 (PF4) and  $\beta$ -TG. Levels of these two mediators are enhanced in the peripheral circulation of IBD subjects even though they do not reflect clinical activity (19, 23, 24). 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. Compared to healthy controls, circulating platelets in both CD and UC patients have significantly greater expression of this potent immunoregulatory and proinflammatory molecule than normal platelets, and this difference persists even after *in vitro* thrombin stimulation (25). These CD40L-positive platelets are essentially the only source of the increased plasma levels

**Table 1.** Abnormalities Indicative of Platelet Dysfunction in IBD

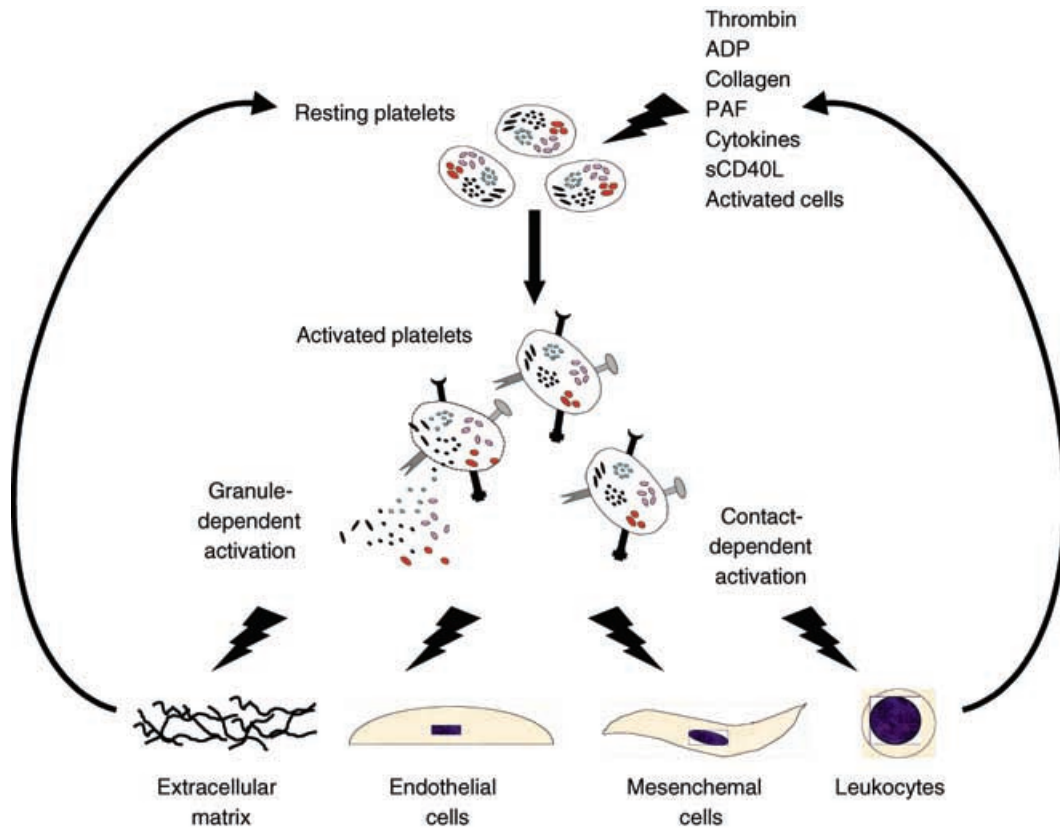
Reactive thrombocytosis (platelet count $>450 \times 10^9/L$ ) (8)
Reduction in mean platelet volume (17)
Increase in granular content (16)
Increase state of activation in the peripheral circulation (19, 25)
Spontaneous aggregation and increased susceptibility to aggregating agents (15, 19)
Frequent occurrence of mucosal microvascular thrombi (59)

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 (26). The abnormalities indicative of platelet dysfunction in IBD are summarized in Table 1.

## PLATELETS AS INFLAMMATORY CELLS

The notion that platelets can function as inflammatory cells is supported by several lines of evidence. First, platelets produce and store enormous amounts of inflammatory mediators; second, platelets simultaneously cross talk with and activate different cells; third, platelets are in turn activated by multiple proinflammatory substances through cognate receptors expressed on their surface (Fig. 1) (3, 4, 6, 27–30).

Platelets' proinflammatory activity is mediated primarily by biologically active molecules kept in intracellular storage compartments represented by the  $\alpha$ -granules and dense body systems (4). Upon activation, a large number of these substances are promptly released in the surrounding microenvironment (Table 2). Among them, histamine, prostaglandin E<sub>2</sub> and D<sub>2</sub>, platelet-derived growth factor (PDGF), thromboxane A<sub>2</sub> and serotonin control vascular permeability and regulate vasodilatation or vasoconstriction (31); adenine nucleotides, PDGF, and PF4 induce neutrophil activation and degranulation; TGF- $\beta$  and basic fibroblast growth factor (bFGF) promote fibroblast proliferation and wound repair while vascular endothelial growth factor (VEGF) is a major trophic factor for endothelial cells and promotes angiogenesis (27, 32–34). In addition, heparanase is also released and causes degradation of subendothelial extracellular matrix, facilitating leukocyte extravasation into and infiltration of the extravascular tissue compartment (35). Of particular importance is the observation that platelets also discharge major chemoattractant molecules, including chemokines regulated on activation normal T-cell expressed and secreted (RANTES), macrophage chemoattractant protein (MCP)-3, growth-regulated oncogene (GRO)- $\alpha$ , and macrophage inflammatory protein (MIP)-1 $\alpha$ , as well as PAF, and the leukotriene 12-HETE (36, 37), all of which contribute to leukocyte recruitment. In addition to these classical proinflammatory molecules, activated platelets secrete 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



**Figure 1.** Contribution of platelets to cellular activation and inflammation. Upon exposure to a variety of cells and soluble factors, resting platelets become activated, express new receptors, and release biologically active molecules stored in their granules. Through granule- and contact-dependent mechanisms, platelets activate both immune and nonimmune cells, which mediate inflammation and lead to further platelet activation.

macrophages, as well as nonimmune cells such as mesenchymal and endothelial cells (38–40). 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 (41, 42). Finally, activated platelets are able to produce IL-1 $\beta$  and IL-7 which, through their numerous and potent biological properties, further widen platelets' range of proinflammatory and immunoregulatory functions (43, 44).

Activation causes major morphological changes in platelets, and their normal discoid appearance is lost due to the development of long pseudopodia with the acquisition of an adhesive phenotype. This dramatic metamorphosis is accompanied by the rapid up-regulation of several adhesion and proinflammatory molecule, including P-selectin, glycoprotein IIb/IIIa, and CD40L and, through contact mediated by these molecules, platelets further amplify their proinflammatory activity by activating surrounding cells (6). For instance, ligation of platelet P-selectin with its counterligand PSGL-1 induces TNF- $\alpha$  and chemokine production by monocytes and CD4<sup>+</sup> T-cells, as well as generation of superoxide free radicals by neutrophils (45–47). Furthermore, P-selectin up-regulation induces the formation of platelet–

leukocyte aggregates, and immobilized platelets support P-selectin dependent-leukocyte rolling, contributing to promote leukocyte adhesion to and transmigration through endothelium and amplification of the local immune responses (48–50). In addition to P-selectin, GP IIb/IIIa also mediates the adhesion of platelets to neutrophils and endothelium, fostering and sustaining the interaction between immune and nonimmune cells (51).

Finally, platelets express surface FC- $\gamma$ RII and IgE, and an increasingly large number of functional receptors for cytokines (IL-1 $\beta$ , TNF- $\alpha$ ), chemokines (CCR1, CCR3, CCR4, CXCR4), and complement components (C1q, C3, C8). Very recently, CD40 has also been found to be constitutively expressed by platelets, where upon binding, directs platelet response towards inflammation rather than aggregation (52). In human diseases, the upregulated expression of these surface receptors presumably contribute to maintenance of inflammation (36, 37, 53, 54).

## INVOLVEMENT OF PLATELETS IN MUCOSAL INFLAMMATION

That platelets become activated and participate in the pathogenesis of several chronic diseases of autoimmune, allergic,

**Table 2.** Major Biologically Active Mediators Released by Activated Platelets

Mediator	Dominant Biological Action Relevant to Inflammation
Histamine	Regulation of vascular permeability (31)
PGE <sub>2</sub> -PGD <sub>2</sub>	Vasodilatation (31)
PDGF	Vasoconstriction, smooth muscle cell proliferation, chemotaxis, leukocyte activation (31)
Thromboxane A <sub>2</sub>	Vasoconstriction, proinflammatory (31)
Serotonin	Vasoconstriction, smooth muscle cell proliferation, chemotaxis (31)
bFGF	Angiogenesis (33)
VEGF	Angiogenesis (34)
Adenine nucleotides	Neutrophil activation and degranulation (27)
Heparanase	Extracellular matrix degradation (35)
TGF- $\beta$	Fibroblast proliferation, wound repair, immunosuppression (32)
PF-4	Chemotaxis, leukocyte adhesion (37)
$\beta$ -thromboglobulin	Chemotaxis, leukocyte activation and adhesion (37)
RANTES	Chemotaxis, T-cell activation, leukocyte adhesion (36)
MIP-1 $\alpha$	Chemotaxis (36)
PAF	Chemotaxis, platelet activation (36, 37)
MCP-3	Chemotaxis, leukocyte activation (36)
GRO- $\alpha$	Chemotaxis, leukocyte activation (36)
12-HETE	Chemotaxis (3)
sCD40L	Proinflammatory, prothrombotic, immunoregulatory (39)
IL-1 $\beta$	Proinflammatory (43)
IL-7	Immunoregulation (44)

and vascular origin is widely acknowledged. In rheumatoid arthritis activated platelets are found in the synovial fluid of the affected joints, while in systemic lupus erythematosus circulating platelets display a significantly elevated expression of activation markers (55, 56). Platelets also have a major pathogenic role in chronic inflammatory coronary syndromes (57), and platelet-derived sCD40L is centrally involved in the febrile response to blood transfusion (58).

The initial suggestion that platelets could also be involved in chronic intestinal inflammation was inferred from histopathological studies revealing the presence of mucosal capillary thrombi in rectal biopsies of patients with IBD (59). 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 (59). These observations were complemented by the finding of an increased expression of the procoagulant molecule tissue factor, which closely correlates with the degree of thrombosis in the mucosal microvasculature of CD patients (60). In fact, one of the earliest abnormalities in CD mucosa is the presence of platelet thrombi cross-linked with fibrin in the mucosal microvasculature (61). This feature, however, is not specific of CD as can be found in other idiopathic inflammatory bowel disorders (60). In reality, the intimate adherence of platelets to the endothelium is a general phenomenon characteristic of

the early manifestations of regional immune reactivity (62), and persists throughout the course of several inflammatory conditions, including IBD (59, 61).

The adherence of platelets to microvascular endothelium is not a simple physical contact, but rather it translates the functional consequence of platelet activation at the tissue level. That platelet activation occurs in IBD mucosa was originally suggested by the finding of increased platelet aggregates in the mesenteric blood of CD patients (22). The same event was recently reproduced *in vitro* using platelets cocultured with human intestinal microvascular endothelial cells (HIMEC). HIMEC pretreated with IL-1 $\beta$  to mimic IBD endothelium can activate platelets through simple physical contact, as evidenced by a sustained up-regulation of P-selectin and CD40L expression on the platelet surface (26).

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 proinflammatory response (25). The *in vitro* counterpart for this finding is the up-regulation 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 promoting 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 (25), 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 (63).

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 (64). 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 (65).

A final 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 (66). 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 (67).

## THERAPEUTIC IMPLICATIONS

Once seen as mere participants of the coagulation cascade, platelets have gained a new status as important components of localized inflammatory responses (68). Because platelet activation contributes to inflammation primarily at the tissue level, targeting this cell type appears to be a logical therapeutic strategy in various disease conditions, including CD and UC (69). Increasing evidence is emerging from the literature in favor of the therapeutic potential of specifically inhibiting platelet function in IBD. Like all salicylic compounds, 5-aminosalicylic acid (5-ASA), a mainstay of IBD therapy, induces a wide array of modulatory activities, which include the inhibition of platelet activation. In fact, platelets isolated from the circulation of IBD patients receiving mesalazine or olsalazine display reduced spontaneous and thrombin-induced platelet activation *in vitro*, as well as low expression of P-selectin *in vivo* (70). The possible mechanism for inhibition of platelet activation by 5-ASA compounds remains to be established, but a plausible explanation is that reduced activation results in less interaction with endothelial and inflammatory cells and, subsequently, diminished release of pro-inflammatory mediators, all of these representing steps that could down-regulate gut inflammation.

When considering inhibition of platelet function as a possible antiinflammatory therapy in IBD, it is critical to dissociate inhibition of platelet aggregation/adhesion from inhibition of platelet activation. Traditional antiinflammatory drugs that inhibit platelet aggregation and prevent thrombosis, such as aspirin and nonsteroidal antiinflammatory substances, are ineffective or even worsen IBD. On the other hand, drugs that selectively turn off the inflammatory potential of platelets may yield real therapeutic benefits to IBD patients. This differential potential is well illustrated by a recent report where the authors attempted to impede the release of activated platelets' sCD40L with aspirin or glycoprotein IIb/IIIa antagonists (eptifibatide, abciximab, and tirofiban) (71). While platelets from patients receiving aspirin only displayed a modest reduction, those from patients given the glycoprotein IIb/IIIa antagonists exhibited a much greater reduction of sCD40L release.

Other drugs that target the inflammatory potential of platelets are ridogrel, a combined thromboxane synthase in-

**Table 3.** Potential Novel Targets for Antiplatelet Therapy

Thromboxane
Glycoprotein IIb/IIIa
Thromboxane and prostaglandins
Arp 2/3 complex
Growth-arrest specific gene 6 (Gas6)
CD39/ATPDase
CD40/CD40L pathway
GP Ib-V-IX complex von Willebrand factor
Platelet leptin receptor
Pepducins
Protease-activated receptors (PARs)
P-selectin
P2Y <sub>1</sub> and P2Y <sub>12</sub> receptors
RANTES

Adapted from Ref. (69).

hibitor and thromboxane/prostaglandin endoperoxide antagonist, and picotamide, a thromboxane synthesis inhibitor receptor antagonist (72). Because ridogrel ameliorates animal models of experimental colitis (73) and platelets are a rich source of thromboxane A<sub>2</sub>, this drug has been used in clinical trials with CD and UC patients, but has been ineffective in improving disease activity (74, 75). Picotamide appears to be effective at least *in vitro* in inhibiting the excess of thromboxane produced in the mucosa of IBD patients (76), but clinical data regarding the clinical efficacy of this compound in UC or CD patients are still not available (77). An important issue to be considered in antiplatelet therapy, particularly in active IBD patients, is the potential risk of bleeding requiring careful dose finding studies and close clinical monitoring. This adverse effect has actually been reported as a complication in UC patients undergoing heparin treatment, even though worsening of rectal bleeding is infrequent and only rarely requires blood transfusion or colectomy (69, 78). A comparison between heparin and the antiplatelet drugs discussed above cannot be properly performed in terms of mechanism of action because heparin, though displaying a slight antiplatelet activity, acts mainly through anticoagulant and antiinflammatory properties. Nevertheless, recent evidence suggests a lack of efficacy of heparin in the treatment of UC (78, 79).

With the continuous advance of our understanding of platelet biology, novel, more specific, and potentially more effective compounds for modulation of platelet function are being developed. In the field of cardiovascular biology, a large number of highly specific drugs are under active investigation to target several of platelet-associated inflammatory molecules (Table 3). The results of clinical trials with some of these compounds will hopefully reveal which, among so many options, could be rationally applied to complement or expand the current therapeutic arsenal of IBD.

## CONCLUSIONS

The intricacies of IBD pathophysiology and the numerous challenges posed by clinical management of difficult CD

or UC patients are widely appreciated. Facing these stumbling blocks, investigators and clinicians alike make every effort to gain additional insights into mechanisms of disease to reach an understanding of why and how chronic gut inflammation comes about, and to develop alternative options to improve treatment of IBD. Although tremendous progress has been made in both areas, new knowledge often leads to new questions and additional challenges. 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 (80, 81), appear to have generated still another challenge. Indeed, because activated platelets are previously unsuspected but active conspirators 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. 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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